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# Effects of Heat and Iron Fractions on Lipid Oxidation in Meat.

Dongmei Han

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**Effects of heat and iron fractions on lipid oxidation in meat**

**Han, Dongmei, Ph.D.**

**The Louisiana State University and Agricultural and Mechanical Col., 1992**

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**EFFECTS OF HEAT AND IRON FRACTIONS  
ON LIPID OXIDATION IN MEAT**

**A Dissertation**

**Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agricultural and Mechanical College  
in partial fulfillment of the  
requirements for the degree of  
Doctor of Philosophy**

**in**

**The Department of Animal Science**

**by**

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**May, 1992**

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## TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS.....	ii
LIST OF TABLES.....	v
LIST OF FIGURES.....	vii
ABSTRACT.....	viii
 CHAPTER	
I. INTRODUCTION.....	1
II. REVIEW OF LITERATURE.....	6
III. EXPERIMENT ONE CHROMATOGRAPHIC DETERMINATION OF HEMOGLOBIN, MYOGLOBIN AND TOTAL PIGMENTS IN BEEF AND CHICKEN MUSCLES.....	33
INTRODUCTION.....	34
MATERIALS AND METHODS.....	36
RESULTS AND DISCUSSION.....	42
IV. EXPERIMENT TWO EFFECT OF HEAT ON THE DISTRIBUTION OF IRON IN BEEF AND CHICKEN MUSCLES.....	54
INTRODUCTION.....	55
MATERIALS AND METHODS.....	57
RESULTS AND DISCUSSION.....	65
V. EXPERIMENT THREE EFFECT OF HEAT AND DIFFERENT FRACTIONS OF IRON ON LIPID OXIDATION .....	77
INTRODUCTION.....	78
MATERIALS AND METHODS.....	81
RESULTS AND DISCUSSION.....	92
VI. SUMMARY.....	116
VII. LITERATURE CITED.....	122

APPENDICES.....	134
VITA.....	161

## LIST OF TABLES

Table		Page
3.1	Least-squares means of total pigment contents with different analytical techniques.....	50
3.2	Least-squares means of Mb and Hb contents in bovine muscle with HIC.....	52
4.1	Iron contents (ug Fe/g sample) in various fractions of bovine longissimus muscle at different temperatures.....	66
4.2	Iron contents (ug Fe/g sample) in various fractions of chicken thigh at different temperatures.....	67
5.1	Concentration of each iron fraction added to beef or chicken model system I and II.....	88
5.2	Least-squares means for percentage composition of fatty acids in beef and chicken model systems (MS).....	93
5.3	Effect of heat and various iron fractions on lipid oxidation expressed as TBA values in water-extracted beef residue (model system I) stored at 4°C for 48h.....	95
5.4	Effect of heat and various iron fractions on lipid oxidation expressed as PV in water-extracted beef residue (model system I) stored at 4°C for 48h.....	96
5.5	Effect of heat and various iron fractions on lipid oxidation expressed as TBA values in water-extracted chicken thigh residue (model system I) stored at 4°C for 48h.....	102
5.6	Effect of heat and various iron fractions on lipid oxidation expressed as PV in water-extracted chicken thigh residue (model system I) stored at 4°C for 48h.....	103
5.7	Effect of heat and various iron fractions on lipid oxidation expressed as TBA values in emulsion of beef intramuscular fat (model system II) stored at 4°C for 48h.....	107



5.8	Effect of heat and various iron fractions on lipid oxidation expressed as PV in emulsion of beef intramuscular fat (model system II) stored at 4°C for 48h.....	108
5.9	Effect of heat and various iron fractions on lipid oxidation expressed as TBA values in emulsion of chicken intramuscular fat (model system II) stored at 4°C for 48h.....	111
5.10	Effect of heat and various iron fractions on lipid oxidation expressed as PV in emulsion of chicken intramuscular fat (model system II) stored at 4°C for 48h.....	112

## LIST OF FIGURES

Figure		Page
3.1	HPLC chromatogram with size exclusion column separation of pigments in a cytochrome c, Hb, and Mb standard solution (A); bovine longissimus (B); and chicken thigh (C).....	43
3.2	HPLC chromatogram with hydrophobic interaction column separation of pigments in a cytochrome c, Hb, and Mb standard solution (A); bovine longissimus (B); and chicken thigh (C).....	44
4.1	Effect of temperature on the iron contents in the water soluble and insoluble fractions in bovine longissimus muscle.....	69
4.2	Effect of temperature on the iron contents in the water soluble and insoluble fractions in chicken thigh.....	69
4.3	Effect of temperature on the contents of diffusate iron in beef longissimus and chicken thigh muscles.....	70
4.4	Effect of temperature on the content of total heme iron in beef longissimus muscle.....	73
4.5	Effect of temperature on the content of total heme iron in chicken thigh.....	73
4.6	Effect of temperature on the contents of hematin iron in beef longissimus and chicken thigh muscles.....	75

## ABSTRACT

Size exclusion (SE) and hydrophobic interaction (HIC) high performance liquid chromatography (HPLC) methods were developed for determining hemoglobin, myoglobin, and total pigments in beef and chicken. Hemoglobin and myoglobin in beef, but not in chicken, were separated by HIC with minimum detectable levels of 0.187 and 0.085  $\mu\text{g}/\mu\text{l}$ , respectively. Determination of total pigment by SE-HPLC (minimum detection of 0.001  $\mu\text{g}/\mu\text{l}$ ) was more sensitive than with spectrophotometric assay.

The effects of heat at 55°C, 70°C, 85°C, and 100°C on the distribution of iron in six fractions (water-soluble, water-insoluble, diffusate, hematin, total heme, and ferritin) in beef and chicken muscles were determined. Iron content decreased ( $p<0.05$ ) in water-soluble fractions and increased ( $p<0.05$ ) in water-insoluble fractions as temperature increased to 100°C. The content of heme iron was decreased more from 55°C to 85°C than from 27°C to 55°C or 85°C to 100°C. The increase in the amount of diffusate iron appeared to be less than the decreased amount of heme iron at each heating temperature level. As temperature increased, hematin iron content was increased ( $p<0.05$ ) and ferritin iron content was decreased ( $p<0.05$ ). More ferritin iron was decreased ( $p<0.05$ ) at temperature of 70°C than at 55°C.

The catalytic abilities of heat at 55°C, 70°C, 85°C, and 100°C and nine forms of iron (water-extractable iron,

diffusate iron, non-diffusate iron, hematin, Hb, Mb, ferritin,  $\text{FeCl}_2$  and  $\text{FeCl}_3$ ) on lipid oxidation in water-extracted meat residues (WR) and solvent-extracted fat emulsion (FE) of beef and chicken muscles were studied. Heat and different forms of iron promoted lipid oxidation ( $p < 0.05$ ) in WR and FE for both species. The catalytic abilities of the same forms of iron were different in WR and FE. All forms of iron catalyzed lipid oxidation in WR, however, ferritin,  $\text{FeCl}_2$ , or  $\text{FeCl}_3$  did not increase lipid oxidation in FE. Heme iron was the major catalyst of lipid oxidation in FE, while both heme iron and iron in the low molecular weight fractions (diffusate iron) had high catalytic activity for lipid oxidation in WR.

## **CHAPTER I**

### **INTRODUCTION**

Lipid oxidation is a major cause of deterioration in the quality of stored meat and meat products. This may directly affect many quality characteristics such as flavor, color, texture, nutritive value and safety. The unsaturated fatty acids, particularly the polyunsaturated fatty acids, are oxidized to hydroperoxides by a free radical chain mechanism involving initiation, propagation, and termination stages (Uri, 1961; Lundberg, 1962; Pearson et al., 1983). Cooked meat is more susceptible to lipid oxidation than uncooked meat during refrigerated storage, resulting in "warmed-over flavor" (WOF) (Tims and Watts, 1958).

Since lipid oxidation is a serious problem in meat, there has been great interest in identifying the catalysts that promote the oxidation of muscle lipids. It is generally accepted that iron in some form catalyzes the oxidation of lipid in meat. Heme pigments, myoglobin (Mb) and hemoglobin (Hb), have generally been considered to be the major catalysts of lipid oxidation in meat, especially in raw red meat (Younathan and Watts, 1959; Tappel, 1962; Greene, 1971; Love, 1983; Rhee, 1988), while a number of researchers have proposed that nonheme iron plays a major role in accelerating lipid oxidation in cooked meat (Sato and Hegarty, 1971; Love and Pearson, 1974; Igene et al., 1979). However, other researchers have attempted to assess the relative importance of heme and nonheme iron as catalysts of lipid oxidation in various animal tissues and concluded that both heme and

nonheme iron have catalytic activity in raw and cooked meat systems (Wills, 1966; Liu and Watts, 1970). In spite of the numerous publications on the subject, the major catalysts of lipid oxidation in meat are still not affirmed.

Iron in muscle can exist in a number of different complexes, including low molecular weight fractions, heme compounds, and storage complexes such as ferritin and hemosiderin (Hazell, 1982; Stryer, 1988). The major source of heme iron in meat is provided by the muscle protein Mb and the blood protein Hb. The Hb content depends on the extent of the vascular bed in the muscle and the bleeding of the carcass (Oellingrath et al., 1990). Quantitative determination of the two heme pigments would be helpful in studying their catalytic effects on lipid oxidation as well as in more accurately evaluating muscle color. Hydrophobic interaction high-performance liquid chromatography (HIC) is a time-saving, accurate protein separation method that uses the mechanism of selective precipitation and solubility in relatively mild salt solutions to isolate and characterize proteins in their native states (Nelson, 1989). Oellingrath et al. (1990) reported that Mb and Hb in beef samples were completely separated by hydrophobic interaction HPLC (HIC). There is great potential for use of HPLC methods in separation of Hb and Mb in muscle since traditional separation and quantification techniques are based only on

differences in chemical compositions, hydrophobicity, or solubility rather than combinations of these differences.

Heat would affect the distribution of iron in various fractions in meats (Schricker et al., 1982; Chen et al., 1984) causing changes in the degree and rate of lipid oxidation in cooked meat. Heat was reported to reduce the heme iron content and increase the nonheme iron content in meat due to the release of iron from the heme iron complex by oxidative cleavage of the porphyrin ring (Schricker et al., 1982; Chen et al., 1984). The increase of nonheme iron in meat due to heat was probably responsible for the more rapid development of lipid oxidation in cooked meat compared with raw meat (Love and Pearson, 1974; Igene et al., 1979). More information on the effect of heat on distribution of different fractions of iron in meat may help elucidate the role of various forms of iron in WOF production.

General objectives of this study were to develop a method to separately measure Mb and Hb from muscle and to determine more specifically the effects of heat and different fractions of meat iron on lipid oxidation. Three experiments were conducted in this study. The first experiment used high performance liquid chromatography (HPLC) to accurately and rapidly determine the contents of total pigments, Mb, and Hb in bovine and chicken muscles. The second experiment was conducted to assay the effect of increased temperatures on the distribution of different fractions of iron in bovine and



chicken muscles. The third experiment examined the role of different forms of meat iron on lipid oxidation in selected model systems prepared from beef or chicken muscle, and to further assess the effect of heating temperature on lipid oxidation in the model systems as catalyzed by different forms of iron.

## **CHAPTER II**

### **REVIEW OF LITERATURE**

### **Composition of meat**

Meat contains water, protein, fat, carbohydrate, and inorganic constituents. Water constitutes approximately 75 percent of the muscle by weight. Water is the principal constituent of extracellular fluid and numerous chemicals are dissolved or suspended in it (Judge et al., 1989). Proteins comprise 16-22 percent of the muscle by weight and they are the principle components of meat solid matter. Based primarily on their solubility, muscle proteins generally are categorized as sarcoplasmic, myofibrillar, or stromal proteins. Sarcoplasmic proteins include myoglobin and enzymes associated with energy metabolism which are soluble in water or low ionic strength buffers. Myofibrillar proteins include the proteins in intracellular myofilaments extracted with intermediate to high ionic strength buffers. Stromal proteins constitute connective tissue and associated fibrous proteins, and they are comparatively insoluble (Judge et al., 1989). In addition to proteins, other nitrogenous compounds are present in muscle, including amino acids, simple peptides, creatine, creatine phosphate, creatinine, some vitamins, nucleosides, and nucleotides such as adenosine triphosphate (ATP) (Judge et al., 1989).

The content of lipid in meat is variable, ranging from approximately 1.5 to 13 percent. Animal fat has been generally categorized into four types: visceral (surrounding vital organs), subcutaneous (beneath the skin), intermuscular

(between muscles), and intramuscular (within muscles) fat (Judge et al., 1989). While some lipid is found intracellularly in muscle fibers (intramuscular fat), the majority of fat in meat is present as intermuscular fat. The contents of the polyunsaturated fatty acids in intramuscular and intermuscular fat were almost the same. However, intramuscular fat was found to contain slightly higher amounts of polyunsaturated fatty acids than subcutaneous adipose fat (Ostrander and Dugan, 1962). The lipids in muscle are primarily composed of triglycerides and phospholipids. Triglycerides are neutral lipids. In chicken, beef, pork, and lamb, the neutral or nonpolar lipids contain about 40-50% monoenoic fatty acids and less than 2% of the most highly unsaturated fatty acids (Allen and Foegeding, 1981). Phospholipids are the main components of the membrane-bound lipids and are characterized by their high levels of polyunsaturated fatty acids (Judge et al., 1989).

Palmitic ( $C_{16}$ ) and stearic ( $C_{18}$ ) acids are the predominant saturated fatty acids in muscle fat. The predominant unsaturated fatty acids in animal fats are palmitoleic, oleic ( $C_{16}$  and  $C_{18}$ , respectively, with 1 double bond), and linoleic ( $C_{18}$ , 2 double bonds). Linoleic is the most abundant polyunsaturated fatty acid in muscle fat (Ostrander and Dugan, 1962; Judge et al., 1989).

Animal lipids are considered to be fairly saturated. The ratio of saturated fatty acids to unsaturated fatty acids

in meat depends on species, the animal's diet and the environmental temperature (Pearson et al., 1977). Generally, the unsaturated fatty acid content in intramuscular fat is in the decreased order of poultry, pork, beef, and lamb (Wilson et al., 1976; Judge et al., 1989). Chicken and pork muscle fat contain more polyunsaturated fatty acids than beef and lamb muscle fat (Lillard, 1987).

The carbohydrate content of muscle is generally quite small. The most abundant carbohydrate in muscle, glycogen, comprises approximately 0.5-1.3 percent of muscle weight. Finally, muscle contains numerous inorganic constituents, such as calcium, magnesium, potassium, iron, phosphorus, and chlorine (Judge et al., 1989).

#### **Forms of iron-containing compounds found in meat**

Iron in muscle can exist in a number of different complexes, including low molecular weight molecules, heme compounds, storage complexes of ferritin and hemosiderin, and transport protein transferrin (Torrance et al., 1968; Hazell, 1982; Stryer, 1988). Iron may be associated with adenosine dinucleotide phosphate (ADP), pyrophosphate, or some amino acids with low molecular weights (Hazell, 1982; Asghar et al., 1988).

The major iron-containing compounds in meat are heme pigments (Love, 1987). Heme compounds in meat include hematin, hemoglobin (Hb), myoglobin (Mb), cytochrome c, and several heme-containing enzymes such as catalase and

peroxidase (Apte and Morrissey, 1987a; Stryer, 1988). Hematin is a free heme having two coordinate water molecules or hydroxyl groups (Ladikos and Wedzicha, 1988). Mb is the major pigment hemoprotein in muscle tissue, followed by Hb (Fleming et al., 1960; Greene and Price, 1975). Both Hb and Mb are composed of the heme moiety, an iron atom surrounded by a porphyrin ring, and polypeptide chains of amino acids, which consist of 140 to 160 amino acid residues (Dickerson and Geis, 1983; Ladikos and Wedzicha, 1988). A molecule of Hb contains four polypeptide chains, two of which are identical (Perutz, 1965). Each polypeptide chain links with a heme moiety. A molecule of Mb, however, has only one heme moiety and one polypeptide chain (Dickerson and Geis, 1983). The iron atom of the heme has four of its coordination sites involved with bonds to porphyrin. In the protein complex, the fifth site is linked to the imidazole nitrogen of a histidine residue of the globin, while the sixth is available for the binding of other ligands (Ladikos and Wedzicha, 1988). The oxidation state of the iron and the type of the ligand bound to the iron center determine the color and reactivity of hemoproteins. Deoxyhemoglobin (DeoxyHb) and deoxymyoglobin (DeoxyMb) lack a ligand in the sixth position and are the purple-red pigments seen when raw meat is first sliced, before the air oxygenates the surface pigments to oxyhemoglobin (OxyHb) and oxymyoglobin (OxyMb) (Judge et al., 1989; Ladikos and Wedzicha, 1988). OxyHb and OxyMb, stable

under high oxygen tension, are the bright red pigments associated with fresh raw meat, and nitric oxide hemoglobin and myoglobin are the pink pigments of cured meat (Greene and Price, 1975; Judge et al., 1989). In all of these pigment forms, iron is in the  $\text{Fe}^{2+}$  state. Methemoglobin (MetHb) and metmyoglobin (MetMb), seen in raw meat that has been stored, and denatured globin ferrihemichrome, the gray-brown pigment of cooked meat, contain iron in the  $\text{Fe}^{3+}$  state (Greene and Price, 1975).

Ferritin is a soluble iron storage protein present in all cells, especially in the liver, spleen, and bone marrow, which has a molecular mass of 450,000 daltons (Linder, 1988). The ferritin molecule consists of a spherical protein shell that encloses an iron 'core', a complex polymer of iron (III) in the form of hydrated oxide and phosphate (Gutteridge et al., 1983). The iron content in ferritin in muscle is dependent on the degree of iron loaded to ferritin at the time of animal sacrifice during slaughter. One ferritin molecule was found to contain up to 4500 atoms of iron(III) when fully loaded with iron (LaCross and Linder, 1980).

The term hemosiderin has been used since the 1880s to describe ferric hydroxide deposited in the tissues in the form of brown granules visible under the microscope after staining by the Prussian blue reaction for ferric iron. Some 40 years ago, it was demonstrated that cells can also store iron in the form of ferritin. It has often been assumed that

hemosiderin represents the iron cores of ferritin remaining in the cell after removal of its protein shell by intracellular proteolytic enzymes (Wixom et al., 1980). In addition to its use as a term for ferric hydroxide granules seen in tissues, the term hemosiderin is also in current use as a convenient name for chemically-measured tissue iron other than heme iron or ferritin iron (Wixom et al., 1980). In fact, this use of the term includes nearly all water-insoluble iron-containing compounds in raw meat (Torrance et al., 1968).

#### **Importance of iron in meat**

The principle functions of iron in living mammals involve oxygen transport within blood and muscle and electron transfer (Linder, 1988). In meat, most of the iron is associated with Mb; a lesser amount is found in Hb (Love, 1988). The content of the heme pigments: Mb and Hb and the oxidation state of the iron in the heme ring determine the color of meat. The bright red color in fresh meat is due to presence of MbO<sub>2</sub> and HbO<sub>2</sub> in which iron is in Fe<sup>2+</sup> state. However, if meat is stored under conditions of low oxygen tension such as partial vacuum or semipermeable membrane packaging, heme pigments in the meat will be oxidized to MetMb and MetHb which are responsible for the undesirable brown color of meat (Judge et al., 1989).

Iron is also the major catalyst for the development of oxidative rancidity in meat (Love, 1988). Although the form



of iron which is mainly responsible for catalysis of lipid oxidation in meat has not been affirmed, both heme and nonheme iron have been reported to have catalytic ability on lipid oxidation in meat model systems (Younathan and Watts, 1959; Liu and Watts, 1970; Apte and Morrissey, 1987b).

Meats, especially red meats, are rich sources of highly available iron, which is the proportion of dietary iron the body can absorb. Several studies have shown that heme and nonheme iron are absorbed into the body by different mechanisms, and that the absorption of heme iron is much higher than that of nonheme iron (Monsen et al., 1978; Mahoney, 1986).

#### **Distribution of iron in meat**

The content of total iron in beef longissimus muscle has been reported to range from 17.43 to 24.5 mg/g wet sample (Doornebal and Murray, 1982; Hazell, 1982; Marchello et al., 1984). The content of total iron in chicken thigh was 8.8 mg/g wet sample (Hazell, 1982). Hazell (1982) and Decker et al. (1989) reported that 4-14% of the water-extractable iron in beef, lamb, chicken, pork, and flounder have molecular weights of less than 12,000 daltons. About 8% of the iron of the soluble fraction (press juice) from mackerel muscle was reported in the fraction with molecular weights less than 10,000 daltons (Decker and Hultin, 1990). Hazell (1982) reported that there were species differences in the distribution of iron among the various fractions. The major

part of iron in beef and lamb was present as heme compounds with 73% and 59% of total iron, respectively, while 47% and 28.5% of total iron were present in heme compounds in pork and chicken, respectively. A higher percentage of iron existed in the insoluble fraction in pork and chicken (45.4% in pork and 58% in chicken). Less than 15% of the total iron was in ferritin in muscle (Hazell, 1982; Apte and Morrissey, 1987a). Seman et al. (1991) reported that the concentration of iron bound to ferritin in freshly slaughtered beef muscle (<24 hr postmortem) ranged from 1.1-2.8  $\mu\text{g Fe/g}$  muscle.

#### **Measurement of heme pigments**

The heme moiety in hemoproteins is not a fixed prosthetic group. Reversible dissociation into heme and apoproteins may be achieved (Rossi Fanelli et al., 1958). However, in both hemoglobin and myoglobin, the affinity of the heme for the protein at neutral pH is very high (Fronticelli and Bucci, 1963), with the heme-globin equilibrium constant estimated to be on the order of  $10^{12}$  -  $10^{15}$  M (Gibson and Antonini, 1963). Dissociation of heme is increased considerably at acid pH (Lewis, 1954). If the pH of a hemoprotein solution is lowered to less than 3, the characteristic linkage of the prosthetic group with the protein is ruptured and the protein is denatured (Lewis, 1954; Ladikos and Wedzicha, 1988). The cleaved heme dissolves in acetone, while the free protein precipitates (Lewis, 1954). The resulting heme has a well defined

spectrum with three absorbance bands in the visible region (512, 540, and 640 nm) and is called acid hematin (Lewis, 1954; Ladikos and Wedzicha, 1988). This property of heme can be used to determine the total heme content in muscle by decreasing muscle pH to less than 3 to denature hemoproteins and release heme. The released heme is then extracted from meat by acetone and the concentration of heme is determined by spectrophotometry (Lewis, 1954).

Separations of Hb and Mb in muscle extracts have been based on differences in solubility in concentrated salt solutions, charge or size between the two molecules, or absorption spectra of various derivatives in the visible region. DeDuve (1948) introduced a method for the differential spectrophotometric determination of Mb and Hb. However, the spectral differences were small and uncertain when Hb content of tissue was large relative to the Mb content (Schuder et al., 1979). Other researchers have developed chromatographic methods, mostly using ion-exchange techniques, to purify and determine Mb or Hb in muscles from different species (Akeson et al., 1960; Brown, 1961; Yamazaki et al., 1964). Warriss (1976) used Sephadex G75 or G50 to separate Hb from Mb in ovine muscle and quantified Hb spectrophotometrically. Schuder et al. (1979) separated Mb from Hb by subunit-exchange chromatography. However, these methods can be tedious and time consuming, and only Mb or Hb can be quantitatively determined. Recently, hydrophobic

interaction chromatography (HIC) has become an increasingly frequent method of the separation and purification of biomolecules. HIC utilizes the mechanism of selective precipitation and solubility in relatively mild salt solutions to isolate and characterize proteins in their native states (Nelson, 1989). Oellingrath et al. (1990) reported that Hb and Mb in beef muscle have been completely separated with HIC.

The difficulties of complete separation of different heme pigments are often bypassed through the measurement of all heme pigments. Several techniques have been described to measure the concentrations of total pigments in muscle extracts. Among these techniques, spectrophotometric analysis is the one most often used by researchers (DeDuve, 1948; Fleming et al., 1960; Warriss, 1979; Águllo et al., 1990). Fleming et al. (1960) determined the concentration of total pigments in beef muscle extracts as cyanmet-compounds at 540 nm. Pigments in poultry tissue are usually more difficult to determine than the ones in pork, beef, and lamb because chicken extracts may be cloudy. In order to obtain a clear extract from chicken, Saffle (1973) heated the chicken extract at 55°C water bath for 20 min to denature some proteins in the extract, therefore partially purifying the pigment solution. Águllo et al. (1990) considered that the turbidity of aqueous extracts of meats was due to the presence of certain lipid and protein fractions and could be

eliminated by adding trichloroethylene. The use of reflectance spectrophotometry to determine total pigment in meat sample was also reported (Stewart et al., 1965; Franke and Solberg, 1971).

The ranges for the concentrations of total pigments, Mb, and Hb reported in the literature are 2.8-5.0 mg/g, 2.0-5.0 mg/g and 0.62-0.68 mg/g, respectively, in bovine muscle (Fleming et al., 1960; Hunt and Hedrick, 1977; Águllo et al., 1990; Oellingrath et al., 1990). The total pigment concentrations reported in chicken dark muscles have ranged from 0.25-0.50 mg/g (Ball, 1986; Saffle, 1973).

#### **Measurement of the content of other forms of iron in meat**

Total iron content in meat can be relatively easily determined on wet ashed samples by atomic absorption spectrophotometry. The wet ashed samples are prepared by digesting meat with a perchloric and nitric acid solution (Schricker et al., 1982). Several methods have been developed to determine the nonheme iron content in meat, including (1) direct digestion with the mixture of hydrochloric acid and trichloroacetic acid (Schricker et al., 1982), (2) chelation of the nonheme iron with EDTA followed by precipitation with trichloroacetic acid (Igene et al., 1979), and (3) chelation of the nonheme iron with EDTA and precipitation with acetone (Chen et al., 1984). Heme iron is calculated as the difference between total iron and nonheme iron with the assumption that only heme iron is insoluble

under the acidic conditions employed in the nonheme iron assay (Igene et al., 1979; Schricker et al., 1982). The reported nonheme iron values for beef muscle vary widely. For example, reported mean values for the nonheme iron content (wet weight basis) of raw beef longissimus dorsi muscle range from 1.80  $\mu\text{g/g}$  muscle (Igene et al., 1979) to 8.4  $\mu\text{g/g}$  (Schricker et al., 1982). Rhee and Ziprin (1987a) indicated that one method (Igene et al., 1979) might underestimate nonheme iron content as a result of insufficient extraction/recovery of nonheme iron proteins (ferritin, transferrin and others) from the muscle, while the other method (Schricker et al., 1982) could overestimate the nonheme iron content of red meats because of the possible breakdown of heme pigments into nonheme iron. To minimize these effects, Rhee and Ziprin (1987a) recommended treating the meat samples with nitrite before incubation with an acid mixture to minimize the breakdown of heme pigments into nonheme iron.

A number of methods have been devised for the determination of ferritin iron and protein concentrations in tissues. These include: (1) chemical determination of ferritin purified from tissue extracts by heat coagulation of most other tissue proteins followed by column chromatography to remove the remaining contaminating protein (Drysdale and Munro, 1965); (2) direct precipitation of ferritin by treating tissue extracts with antiferritin sera (Leslie and

Kaldor, 1970); and (3) measurement of ferritin by radial immunodiffusion (Bjorklid and Helgeland, 1971). The most commonly used method in the determination of ferritin iron content in muscle is gel filtration chromatography (Hazell, 1982; Decker and Welch, 1990). Ferritin in muscle extract is separated from other water soluble proteins by gel filtration chromatographic techniques, followed by determination of the iron content in the separated ferritin fraction by atomic absorption spectrophotometry.

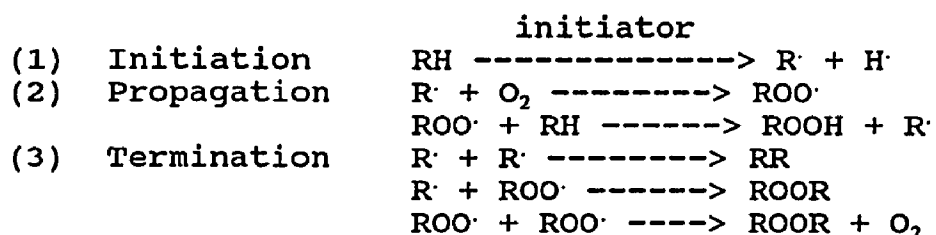
#### **Effect of Heat on the Distribution of Iron in Meat**

Cooking affects the distribution of iron in muscle foods. Several studies have showed that heat reduces the heme iron content and increases the nonheme iron content in meat. Igene et al. (1979) reported that heating a meat extract increased the nonheme iron from 8.7 to 27% of the total amount of iron. Schricker and Miller (1983) found that the nonheme iron increased by 14.63% and 29.27% compared with the original nonheme iron content in ground beef after baking in a 176°C oven for 20 and 40 minutes, respectively. The content of nonheme iron in restructured beef roasts containing either 0 or 3.5% surimi was increased with increasing end-point cooking temperatures from 50°C to 70°C with 10°C intervals (Tanchotikul et al., 1989). By using reversed-phase iron-pair HPLC to determine the content of heme, Oellingrath (1988) reported that heme iron decreased 22% and 30% in MetMb and MetHb solutions, respectively,

heated at 100°C for 1 hour. These increases in nonheme iron may have been due to the release of iron from the heme iron complex by oxidative cleavage of the porphyrin ring (Schricker et al., 1982). The optimal temperatures for the release of iron are between 62°C and 73°C (Chen et al., 1984). Slow heating has resulted in a larger increase in nonheme iron content than that with fast heating in muscle pigment extracts (Chen et al., 1984). The reason for this may be that the rapid increase of temperature in the extract induced coagulation of the hemoprotein before the heme iron had an opportunity to be cleaved from the globin moiety (Chen et al., 1984).

#### Mechanism of Lipid Oxidation

The oxidation of unsaturated fatty acids has been proposed to occur by a free-radical chain mechanism involving the following stages (Uri, 1961; Lundberg, 1962):



The formation of the free radical stage is called initiation. The RH represents any unsaturated fatty acid that in the presence of an initiator loses a labile hydrogen from a carbon atom adjacent to a double bond to form a free radical (R·). The propagation stage involves free radical reactions; the free radical reacts rapidly with O<sub>2</sub> to form a peroxy



radical ( $\text{ROO}\cdot$ ) which reacts with another unsaturated lipid to form a hydroperoxide ( $\text{ROOH}$ ) and a new free radical which can continue the chain reaction. The chain reaction can be terminated by the formation of nonradical products (termination). Because the alkyl radical ( $\text{R}\cdot$ ) reacts rapidly with oxygen to form the peroxy radical, the rate of the  $\text{R}\cdot + \text{O}_2 \rightarrow \text{ROO}\cdot$  reaction is rapid, and most of the free radicals are in the form of the peroxy radical. Consequently, the major termination takes place via the reaction of peroxy radical combination (Wong, 1989). The hydroperoxides, generally called peroxides or primary products of oxidation, are very unstable and can break down to different carbonyls such as malondialdehyde and hexanal, alcohols, hydrocarbons, ester, furans and lactones; all are responsible for the oxidized flavor of meats (Pearson et al., 1983; Ladikos and Lougovois, 1990). The peroxides can also react with proteins, vitamins, and pigments which lead to losses of nutritional value and color changes in meats (Karel, 1973; Gray, 1978).

#### **Role of heme compounds on lipid oxidation**

Heme compounds have been implicated in many studies as playing an important role in catalysis of lipid oxidation in muscle (Younathan and Watts, 1959; Greene, 1971; Love, 1983; Rhee and Ziprin, 1987b; Rhee, 1988). Lipid oxidation in raw water-washed muscle systems increased with increased concentration of Hb to 3 mg/g, while in heated water-washed muscle systems, the pro-oxidative activity increased as the

concentration of Hb increased to 10 mg/g (Apte and Morrissey, 1987b). Raw beef was found to be more susceptible to lipid oxidation than raw pork because there was a higher concentration of heme pigments in beef muscle than in pork muscle (Rhee and Ziprin, 1987b). The most likely mechanism for heme-catalyzed lipid oxidation has been the formation of a coordinate complex between the heme compound and lipid peroxide, followed by homolytic scission of the peroxide bond to generate new free radicals (Love, 1983; Wong, 1989). Recently, activated metmyoglobin was reported to be possibly responsible for the initiation of lipid oxidation in raw red meats (Rhee, 1988). Metmyoglobin was activated by hydrogen peroxide which was generated by the autoxidation of oxymyoglobin with iron in the ferrous state, to metmyoglobin with a ferric iron state in red meats.

The effects of the states of iron in heme on lipid oxidation have been investigated by many researchers. Younathan and Watts (1959) found that uncured cooked meat containing ferric globin hemochromogen had increased lipid oxidation shortly after cooking compared with lipids in cured meat with pigments in the ferrous state and proposed that the ferric form of the pigment was the active catalyst in tissue rancidity. However, Brown et al. (1963) reported that hemes with iron in either the  $\text{Fe}^{2+}$  or  $\text{Fe}^{3+}$  states were effective catalysts of lipid oxidation. Hirano and Olcott (1971) also reported that rates of lipid oxidation were not different

when  $\text{Fe}^{2+}$  or  $\text{Fe}^{3+}$  hemes were promoting lipid oxidation. In a review, Greene and Price (1975) concluded that either  $\text{Fe}^{2+}$  or  $\text{Fe}^{3+}$  hemes might function as catalysts of lipid oxidation, but that  $\text{Fe}^{3+}$  hemes may be necessary for rapid oxidation.

Free hematin was reported to be a more active catalyst of lipid oxidation than methemoglobin, which was more active than metmyoglobin. Oxidized cytochrome c had an oxidative activity between those of methemoglobin and metmyoglobin (Kaschnitz and Hatefi, 1975). However, Sliberstein and Lillard (1978) reported that myoglobin was a more effective catalyst of lipid oxidation in oleic acid emulsions than was hemoglobin. The contradictory and confusing results obtained by different authors may be due to the use of different types of model systems and techniques for following lipid oxidation (Love, 1983). For example, Haurowitz et al. (1973) reported that fatty acids or their methyl esters interact with heme, hemoproteins, or  $\text{Fe}^{2+}$  and  $\text{O}_2$  only at the lipid-water interface in a monomolecular film. No effect of these catalysts was noted in homogeneous systems (Love, 1983).

Fox and Benedict (1987) reviewed studies in the area of the role of heme pigments in lipid oxidation and concluded that heme pigments were not catalytically active in the native protein form. The heme pigments are required to have some degree of denaturation and exposure of the heme for the contact between hydroperoxy lipid and the heme. This viewpoint is supported by hematin being the most reactive

catalyst with the lowest lipid to heme ratio for maximal activity of all the heme compounds studied (Fox and Benedict, 1987).

A complicating factor in the understanding of the catalysis of lipid oxidation by hemes is that high concentration of heme compounds results in the inhibition of oxidation. A number of workers studied the effect in detail (Kendrick and Watts, 1969; Hirano and Olcott, 1971; Lee et al., 1975). Although there was some variation in the reported concentrations, in general heme pigments at or below  $10^{-6}$  M catalyze lipid oxidation and concentrations at or above  $10^{-5}$  M inhibit lipid oxidation (Fox and Benedict, 1987). Unfortunately, the concentration for the antioxidant effect of heme compounds is much higher than the concentration of heme normally reported for meat products.

#### **Role of other iron forms on lipid oxidation in meat**

Meat contains other forms of iron in addition to heme iron. Nonheme iron (inorganic iron or iron in the low molecular-weight fraction) was reported to accelerate lipid oxidation in meat, especially in cooked meat (Sato and Hegarty, 1971; Love and Pearson, 1974). It catalyzed lipid oxidation by joining with thiols or NADPH cytochrome P-450 reductase to initiate lipid oxidation, or by catalyzing the decomposition of lipid peroxides to produce new peroxy radicals in propagation steps (Asghar et al., 1988; Wong, 1989). The form of iron was as important as the amount of

metal present (Ladikos and Lougovois, 1990). Ferrous iron has been shown to have greater pro-oxidant activity than ferric iron in cooked meats (Brown et al., 1963; Wills, 1965; Sato and Hegarty, 1971; Pearson et al., 1977).

Since both heme compounds and nonheme iron have been implicated as pro-oxidants, many researchers have tried to assess the relative importance of heme pigments and nonheme iron as catalysts of lipid oxidation in various animal tissues. Heme pigments were reported to be the major catalysts in raw red meats (Rhee and Ziprin, 1987b; Rhee, 1988), while nonheme iron played a major role in acceleration of lipid oxidation in cooked meat. Work by Sato and Hegarty (1971) and Love and Pearson (1974) revealed that inorganic iron was responsible for rapid oxidation in cooked meat, while heme compounds had little influence on the development of off-flavors in cooked meat. On the other hand, a number of researchers (Wills, 1965; Liu, 1970a,b; Liu and Watts, 1970, Tichivangana and Morrissey, 1985) presented evidence that both heme and nonheme iron could function as pro-oxidants in meats. Catalysis by nonheme iron was reported to be pH sensitive, with iron more active at acid pH values, whereas pH had less effect on hemoprotein-catalyzed lipid oxidation (Wills, 1966).

Pure ferritin was ineffective as a catalyst in raw meat. However, it became an effective catalyst for lipid oxidation in heated muscle systems and in systems where iron was

released from ferritin by the action of ascorbic acid or reducing agents (Wills, 1966; Apte and Morrissey, 1987a). The catalytic activity of ferritin fractions towards lipid oxidation depended on the degree of iron-loading, with higher iron contents more stimulatory to lipid oxidation than lower iron contents (Gutteridge et al., 1983; Halliwell and Gutteridge, 1984)

By using gel filtration chromatographic techniques, Apte and Morrissey (1987a) separated the soluble iron fraction from muscle into ferritin, heme, and dialyzable fractions. The relative contributions of each fraction to lipid oxidation in water-extracted cooked muscle systems were dialyzable fraction about 45%, heme fraction about 33%, and ferritin contributing between 18-25% of total thiobarbituric acid (TBA) values depending on the species used to prepare the muscle system. The insoluble hemosiderin did not play any significant role in the lipid oxidation of muscle.

#### **Effect of heat on lipid oxidation in meat**

A number of researchers reported that heat accelerated the development of oxidative rancidity in meat and meat products (Tims and Watts, 1958; Keller and Kinsella, 1973; Jantawat and Dawson, 1977). The term "warmed-over flavor" (WOF) was used to describe the rapid development of oxidative rancidity in refrigerated cooked meats, in which the rancid flavor became apparent within 48 hours when stored at 4°C (Tims and Watts, 1958).

The intensity of heat treatment would affect the extent of lipid oxidation in various cooked meat. Keller and Kinsella (1973) observed increases in TBA values on cooking of ground beef from room temperature to 70°C. Pearson et al. (1977) reported that meats heated at 70°C for 1 hr developed rancidity rapidly compared to unheated samples. However, TBA values were decreased compared to those at 70°C when the cooking temperature was raised above 80°C. The increased susceptibility of cooked meat to oxidation has been attributed to the following possible factors: (1) the denaturation of lipoproteins in the microsomes and mitochondria and the disruption of the muscle membrane system by heat, which expose more labile lipid components to oxygen and other reaction catalysts (Sato and Hegarty, 1971; Dawson and Gartner, 1983); (2) the denaturation of heme proteins during cooking increased the exposure of catalytic heme group to unsaturated fatty acids (Greene and Price, 1975; Igene et al., 1979).

Meat subjected to high temperatures and/or long periods of heating developed lower TBA values than samples subjected to lower temperatures and/or shorter periods of time (Huang and Greene, 1978). Antioxidants which retard the development of WOF were formed when meat was heated to 80°C or higher (Lillard, 1987). A nonenzymatic browning reaction (Maillard reaction) between amino acids or proteins and carbohydrates resulted in the production of antioxidants in high

temperature treated meat (Sato et al., 1973; Bailey et al., 1987). Several researchers indicated that the Maillard reaction products (MRP), such as melanoidins and their precursors reductones, had strong antioxidant properties in cooked meats (Hamm, 1966; Sato et al., 1973; Huang and Greene, 1978). According to Hamm (1966), the Maillard reactions in meats began at about 90°C and increased with increased temperature and heating time. The most important mechanisms proposed for the antioxidant activity of MRP include the reduction of hydroperoxides to products which are not able to form free radicals, inactivation of free radicals formed during oxidative degradation of unsaturated fatty acids and the complexing of heavy metal ions by the reaction products (Bailey et al., 1987).

#### **Effect of fatty acid composition on lipid oxidation in meat**

The nature, proportion, and degree of unsaturation of fatty acids present in a meat will indicate the approximate susceptibility of the product to oxidative deterioration. Generally speaking, the higher the proportion and degree of unsaturation of the fatty acids, the more labile the lipid system is to oxidation (Dawson and Gartner, 1983). Phospholipids, the main components of the membrane-bound lipids, are characterized by their high levels of polyunsaturated fatty acids and are very susceptible to oxidative rancidity (Igene and Pearson, 1979; Cross et al., 1987). Pork and poultry have greater amounts of



polyunsaturated fatty acids when compared with beef and lamb (Lillard, 1987). Therefore, the potential for WOF development in pork and poultry is greater than in beef and lamb. In fact, poultry is most susceptible to rancidity development, followed by pork, beef and mutton (Wilson et al., 1976). Intramuscular fat of meat animals contains slightly higher amounts of polyunsaturated fatty acids than subcutaneous adipose fat (Ostrander and Dugan, 1962). The intramuscular fat cells also are in close proximity to the lean tissue, and thus could be expected to be more important in the development of rancidity than other types of adipose tissue (Cross et al., 1987).

#### **Measurement of the extent of lipid oxidation**

A variety of methods, both objective and subjective, have been developed to measure the extent of lipid oxidation in meat products. The objective methods measure either primary or secondary oxidation products of unsaturated fatty acids. Of the various methods reported, the TBA test has been the most widely used method (Melton, 1983). The test is based on the spectrophotometric determination of the extracted malondialdehyde and can be performed directly on the food product, followed by extraction of the colored complex (Sinnhuber and Yu, 1958); on an extract of the food (Witte et al., 1970); or on a portion of the steam distillate of the food (Tarladgis et al., 1960). The method using a steam distillate has been the most popular one for measuring

the TBA value, as milligram of malondialdehyde per kilogram of sample, in muscle foods (Melton, 1983; Ladikos and Lougovois, 1990). Malondialdehyde is a three-carbon dialdehyde which is the secondary oxidation product of polyunsaturated fatty acids (Pearson et al., 1983). The distillate method must be modified for cured meat products that contain nitrite. Nitrite reacts with malondialdehyde during the distillation step to form oxime which lowers the TBA number. Sulfanilamide should be added at the blending stage of the distillate method to form diazonium salts with nitrite and prevent the interference (Zipser and Watts, 1962; Melton, 1983).

Other methods reported to measure the degree of lipid oxidation in foods are iodometric technique, oxygen uptake method, and determination of the secondary oxidation products, such as carbonyl, hydrocarbons and fluorescent products (Melton, 1983; Ladikos and Lougovois, 1990). Iodometric technique is a method used to measure peroxide value in foods (AOAC, 1973). However, this method may not be useful as a measure of lipid oxidation in muscle foods during prolonged storage, especially if the muscle is ground, because of the decomposition of peroxide (Melton, 1983). The oxygen absorption or uptake method has been used to follow lipid oxidation in muscle tissue homogenate (Fischer and Deng, 1977) and has also been recommended as the only method for following lipid oxidation in freeze-dried muscle foods

(Melton, 1983). Gas chromatographic techniques have also been developed to successfully detect the secondary oxidation products, such as hexanal and other aldehydes, of lipid in oil and foods (Fritsch and Gale, 1977).

Sensory evaluation of warmed-over flavor (WOF) in muscle foods by trained panels have been used by many researchers (Huang and Greene, 1978; Igene and Pearson, 1979; Younathan et al., 1980; Igene et al., 1985; Poste et al., 1986; Tanchotikul et al., 1989; Arganosa et al., 1989). There is a wide variation in reported methods for sensory evaluation of WOF (Melton et al., 1987; Love, 1988). Some of the variations include use of 4 to 11-point scales or a horizontal line scale to estimate the intensity of oxidized flavors; use of 3 to 10 trained or untrained panelists; evaluation of flavor, aroma, or both; and terminology variations, i.e. warmed-over flavor/odor, rancid flavor/odor or off-flavor/odor (Poste et al., 1986; Melton et al., 1987; Love, 1988). It is very possible that some of the terms used in one study may have described the same flavor attribute of another study. Igene and Pearson (1979) stated that use of a trained panel was probably the best way to measure WOF, but even then it was not clearly established that a panel could differentiate between WOF and other types of autoxidative changes.

Many researchers use both objective and sensory analysis to evaluate the WOF in muscle foods. Significant

correlations between TBA values and off-flavor formation in cooked meats have been reported (Zipser et al., 1964; Poste et al., 1986). Tanchotikul et al. (1989) found that the correlation coefficient between TBA value and WOF scores from sensory evaluation in restructured beef roasts was -0.60. Younathan et al. (1980) reported that a high correlation existed between TBA value and panel scores for cooked ground beef, but not for cooked ground turkey. A correlation coefficient of -0.87 was reported between TBA numbers and sensory scores of warmed-over flavor for cooked chicken white and dark meat (Igene et al., 1985). Jeremiah (1980) used peroxide value (PV) to investigate the lipid deterioration in frozen pork in different types of packaging wrap. Significant relationships were reported between PV and flavor rancidity score for the fresh pork samples but not for the cured pork samples. Palmer et al. (1975) reported that a high PV was detected in turkey meat stored at -28.9°C for 8 months, however, sensory panelists did not detect major flavor changes in the turkey samples.

### **CHAPTER III**

#### **EXPERIMENT ONE: CHROMATOGRAPHIC DETERMINATION OF HEMOGLOBIN, MYOGLOBIN AND TOTAL PIGMENTS IN BEEF AND CHICKEN MUSCLES**

## INTRODUCTION

Muscle and meat color are mainly determined by the concentrations and chemical states of two pigments: myoglobin (Mb) and hemoglobin (Hb) (Fennema, 1985; Judge et al., 1989). Heme pigments also catalyze lipid oxidation in meat (Rhee, 1988), with Mb a more effective catalyst than Hb (Sliberstein and Lillard, 1978). Previous studies on the separation of Mb and Hb in muscle extracts have been based on differences in solubility with concentrated salt solutions, charge or size between the two molecules, or absorption spectra of various derivatives in the visible region.

Águllo et al. (1990) suggested that turbidity in aqueous extracts of meat which caused inaccurate spectrophotometric absorbance measurements of pigments (Fleming et al., 1960; Saffle, 1973; Warriss, 1979) could be eliminated by adding trichloroethylene. Reflectance spectrophotometry has also been used to determine total pigment concentration in meat samples (Stewart et al., 1965; Franke and Solberg, 1971). DeDuve (1948) introduced a method for the differential spectrophotometric determination of Mb and Hb. However, the spectral differences were small and uncertain when Hb content of tissue was large relative to the Mb content (Schuder et al., 1979). Chromatographic iron-exchange, gel filtration, or subunit-exchange techniques have been developed to separate and quantitate Mb or Hb in muscles from different species (Akeson et al., 1960; Brown, 1961; Yamazaki et al.,

1964; Warriss, 1976; Schuder et al., 1979). However, these methods were relatively time consuming and could not quantitate Mb and Hb simultaneously. Oellingrath and Slinde (1985) attempted to use high performance liquid chromatography (HPLC) with gel filtration column to separate Mb and Hb in meat loaves, but the separation was incomplete.

Hydrophobic interaction high performance liquid chromatography (HIC) utilizes selective precipitation and solubility in relatively mild salt solutions to isolate and characterize proteins in their native states (Nelson, 1989). Oellingrath et al. (1990) reported that Mb and Hb in minced beef samples were separated completely by the HIC method. The objectives of this study were to separate Mb and Hb in beef and chicken muscles by HPLC techniques and to compare quantitation of total pigments by HPLC methods with traditional spectrophotometric techniques.

## MATERIALS AND METHODS

### Preparation of samples

The beef samples in this study were longissimus dorsi and psoas major muscles (pH 5.7, short loins, first to sixth lumbar vertebrae) from the left sides of five beef carcasses slaughtered at the Louisiana State University Agricultural Center Meat Laboratory. Approximately 200 g longissimus dorsi and 200 g psoas major muscle from each carcass was obtained from the laboratory. The chicken samples in this experiment were drumsticks (pH 6.45) and thighs (pH 6.3) from three commercial broilers (7 weeks of age) obtained from the Department of Poultry Science, Louisiana State University. Thighs and drumsticks (with bone in) were cut from the whole chicken immediately with a knife as they were brought to the research lab. Muscle samples from the same location from each cattle or chicken were vacuum-packaged individually in vinyl bags and stored at  $-18^{\circ}\text{C}$  for one month.

The frozen samples were thawed at room temperature ( $27^{\circ}\text{C}$ ) for two hours and then trimmed of visible fat and connective tissue with a knife and ground twice through a 4.7 mm stainless steel plate. About 5 g ground sample from each cattle or 15 g from each chicken was randomly selected and homogenized with 15 ml of 0.01 M orthophosphate buffer, pH 6.3, in an Osterizer (Oster, Division of Sunbeam Corporation, Milwaukee, Wisconsin) for 2 minutes. The homogenate was transferred to a 50 ml centrifuge tube and centrifuged at



2000 x g at 4°C for 15 minutes. The top solution was decanted into a 50 ml beaker, while the residues were re-extracted with 10 ml of phosphate buffer and centrifuged. All supernatants were combined in the 50-ml beaker and filtered (No.3 filter paper, Whatman International Ltd., Maidstone, England) into another 50-ml beaker. The extract was further filtered through a 0.2  $\mu$ m membrane (Gelman Sciences Inc., Ann Arbor, Michigan) before chromatographic analyses. The pH value of the meat sample was measured with a portable pH meter (TempMeter 4 Corning Glass Works, Corning, NY) by inserting a glass pH-electrode directly into the ground muscle.

#### **Spectrophotometric determination of total pigment**

A modified procedure described by Fleming et al. (1960) was used to determine the content of total pigment in meat samples. The pigment in a 10-ml aliquot of the supernatant was converted to the cyanmet-form by adding 1 ml of stock solution which contained 1.98 g of potassium ferricyanide and 0.52 g of potassium cyanide per 100 ml of water. In order to obtain a clear solution, the mixture was centrifuged at 2000 x g for 15 minutes and filtered through a 0.2  $\mu$ m membrane filter before absorbance was read spectrophotometrically at 540 nm (Bausch & Lomb Spectronic 70, Milton Roy Company, Rochester, NY).

## Chromatography

The high performance liquid chromatography (HPLC) system (Beckman, Inc., Fullerton, CA) had two solvent metering pumps (Model 110A), a sample injection valve (Model 210A), a system controller programmer (Model 420), and a variable wavelength photo detector (Model 165). All chromatograms in this experiment were recorded and integrated by using a integrator (SP4270, Spectra-Physics, San Jose, CA). Peak areas were used for quantitative calculations of pigment concentrations. The measurement unit of the peak area reported from the integrator was millivolt per second.

The HPLC determination of total pigment was performed with a sample injection of 20  $\mu$ l on a size exclusion column (Spherogel TSK 2000SW, 7.5 mm x 30 cm, Beckman/Altex, San Ramon, CA). The system mobile phase of 0.01 M phosphate buffer and 0.15 M NaCl, pH 6.3 was pumped at a flow rate of 1.0 ml/minute at ambient temperature. The eluant was monitored at 280 nm with sensitivity of 0.05 absorbance units full scale (AUFS).

The separation of Mb and Hb on a hydrophobic interaction column (Hydrophase HP-butyl column, Interaction Chemical, Inc., Mountain View, CA) used system mobile phases of 0.1 M sodium phosphate plus 1.8 M ammonium sulfate, pH 6.8, (solvent A) and 0.1 M sodium phosphate, pH 6.8, (solvent B) with 0.25 g/L sodium azide to prevent bacterial growth. A 10-minute linear gradient from 100% A to 100% B was used and

the eluant was monitored at 280 nm with a flow rate of 1.0 ml/minute and sensitivity of 0.05 AUFS at ambient temperature. Sample injection volume was 20  $\mu$ l.

To determine the minimum detectable level of Mb and Hb by HPLC, the standard Mb and Hb were injected onto the chromatographic columns in decreased concentrations. The lowest concentrations of the standard Hb and Mb that could be detected by the HPLC were defined as the minimum detectable levels of Mb and Hb by HPLC in this experiment.

In order to determine the recoveries of pigments from the HPLC system, both Mb and Hb standard solutions with 5 mg/ml concentration were prepared. The prepared standard Mb or Hb solution was injected onto the chromatographic column, and the eluted Mb or Hb fraction was then collected. Mb and Hb in both of the standard and HPLC-eluted solutions were converted to cyanmet-form by adding cyanide stock solution. The concentrations of Mb and Hb were then determined by spectrophotometry as described previously. The recovery of pigment from HPLC was calculated as the ratio of optical density of Mb or Hb in the standard solution to optical density of Mb or Hb in HPLC-eluted fraction.

### **Standards**

The method developed by Yamazaki et al. (1964) was employed to purify Mb from bovine muscles. The purified Mb was used as the standard for the quantification of Mb and total pigment in bovine muscles. Pure Mb from chicken muscle

was difficult to obtain because of its low concentration. Therefore, commercial horse skeletal Mb (2x crystallized; dialyzed and lyophilized, Sigma Chemical Co., St. Louis, MO) was used as the standard for the determination of total pigment concentration in chicken muscles.

The bovine Hb and cytochrome c standards used in this experiment were commercial preparations (2x crystallized; dialyzed and lyophilized, Sigma Chemical Co., St. Louis, MO). The concentrations of Mb, Hb, and total pigments in the samples were calculated from the standard curves of their corresponding standards.

#### **Statistical Procedures**

Randomized block design (RBD) was used in the experiment to determine differences in concentrations of Mb or Hb between the longissimus dorsi and psoas major muscles in beef and between drumstick and thigh in chicken. The General Linear Model (GLM) procedures of SAS (1985a) were used to analyze the data with type of muscle as the main effect, and the animal (cattle or chicken) as the block.

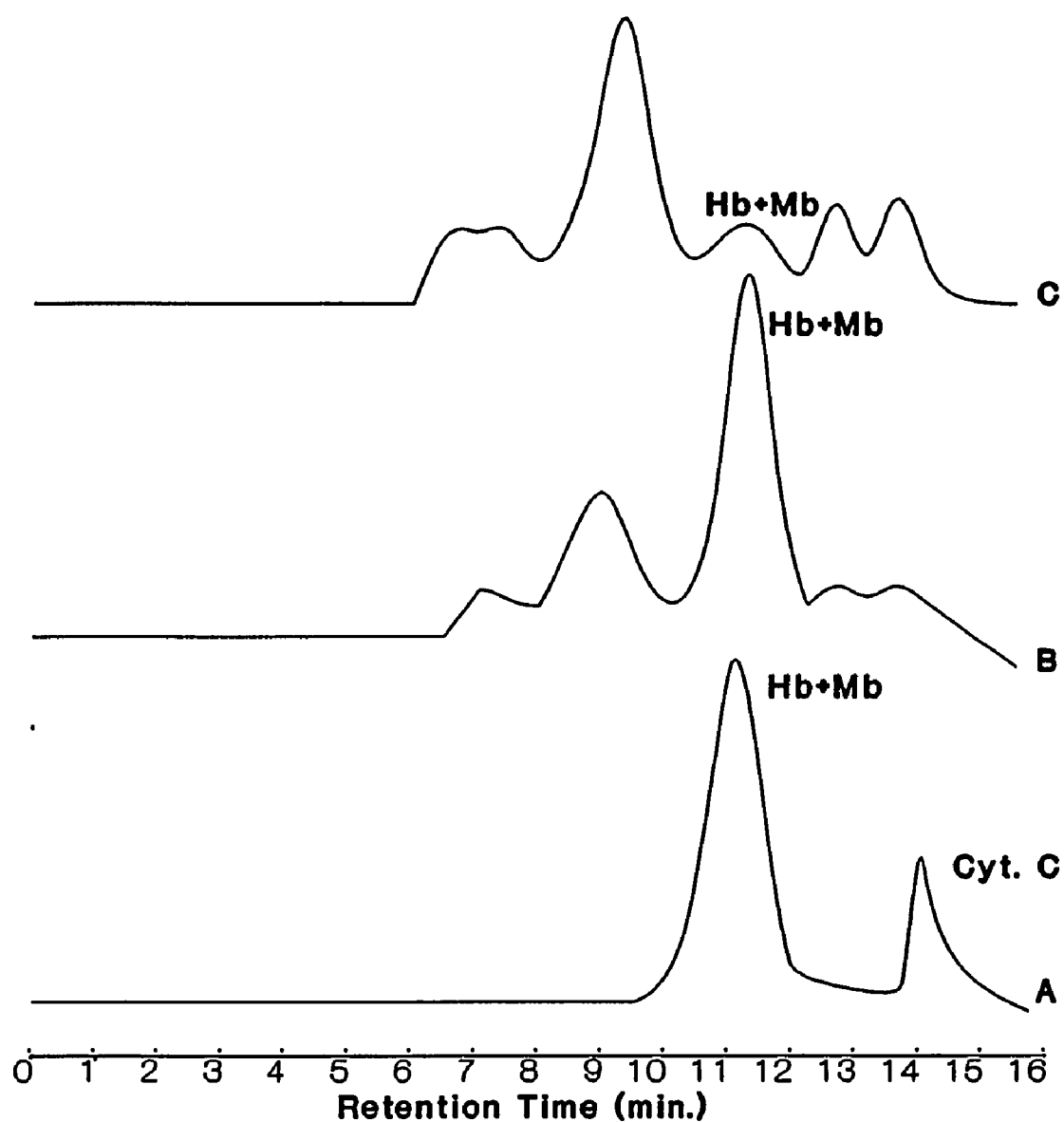
Because three analytical methods were used in the determination of total pigments, a randomized block split-plot design was used to determine whether the concentration of total pigments was different between the type of muscle in beef or chicken and among the three analytical methods. The General Linear Model (GLM) procedures of SAS (1985a) were used to analyze the data from the design with the cattle or

chicken as the block, the type of muscle as the whole plot, and analytical method as the sub-plot. Least-squares means were compared using t-tests when analysis of variance indicated differences in treatments at  $\alpha = 0.05$ .

## RESULTS AND DISCUSSION

### Separation of Mb and Hb

Injection of a standard solution of cytochrome c, Mb and Hb on the size exclusion (SE) column resulted in separation of cytochrome c from Mb and Hb, which eluted as a single peak (Figure 3.1A). Thus, the total concentrations of Mb and Hb in beef and chicken could be measured by calculating the area of a single peak with the use of this column (Figures 3.1B and 3.1C). Standard heme pigments had a retention time of 11.30 minutes, while the retention of standard cytochrome c was 14.06 minutes (Figure 3.1A). Several peaks were shown in the beef and chicken HPLC chromatograms (Figures 3.1B. and 3.1C) including two peaks with the retention time of 9 minutes and 11.30 minutes, respectively. Injection of Mb, Hb or the combination of Mb and Hb standards all produced peaks at a retention time of 11.3 minutes but did not produce any peaks at a retention time of 9 minutes (The chromatogram of solutions containing only the Mb or Hb standard is not shown in Figure 3.1 because both of them produced the identical peak at 11.30 minutes). Therefore, the peaks with retention times of 9 minutes shown in Figures 3.1B and 3.1C did not correspond to either a Hb or Mb peak even though the shapes of the peaks appeared to be similar to the one produced by the Mb and Hb standard. Rather the peaks with retention time of 11.3 minutes were the most likely ones produced by Mb and Hb compounds in the beef and chicken samples. The recovery



**Figure 3.1** HPLC chromatogram with size exclusion column separation of pigments in a cytochrome c, Hb, and Mb standard solution (A); bovine longissimus (B); and chicken thigh (C).

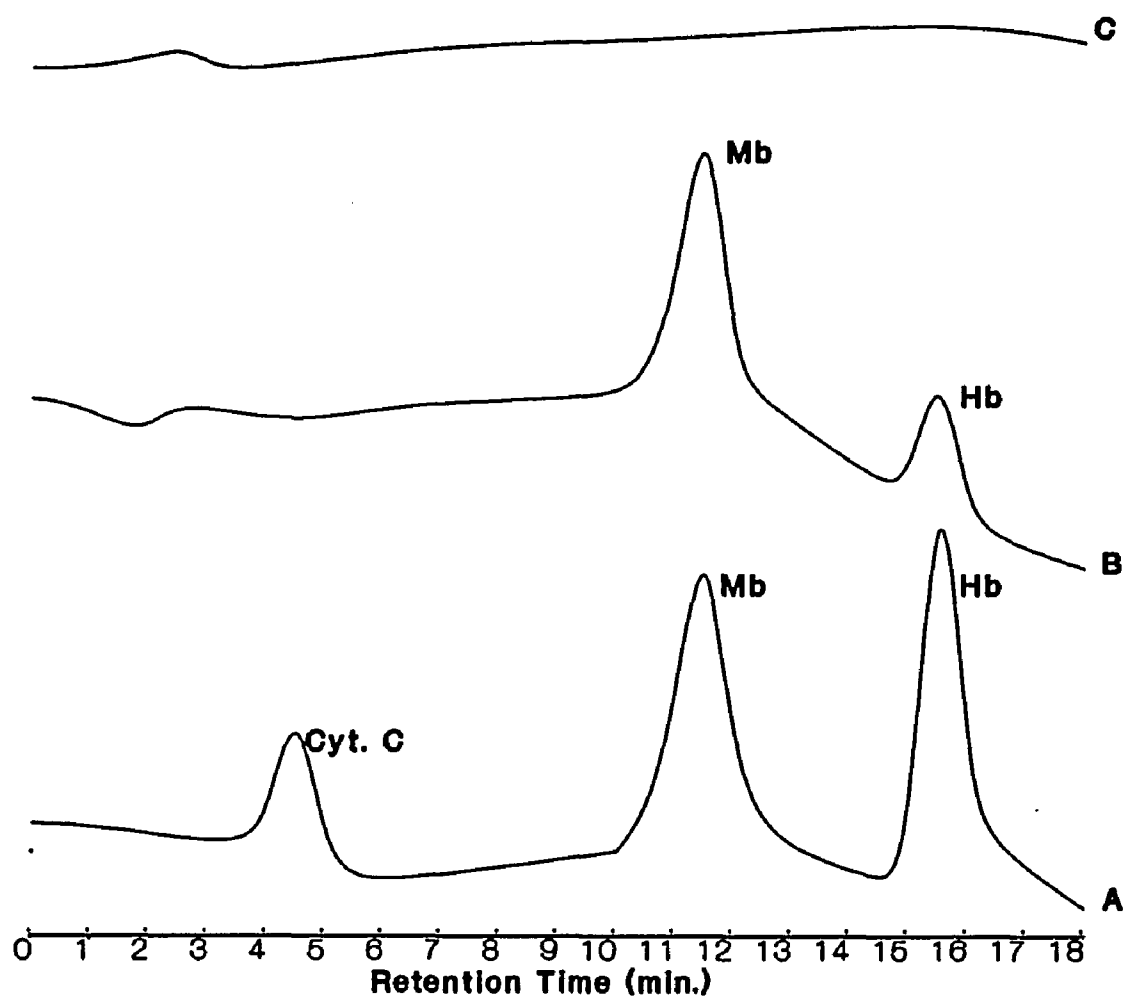


Figure 3.2 HPLC chromatogram with hydrophobic interaction column separation of pigments in a cytochrome c, Hb, and Mb standard solution (A); bovine longissimus (B); and chicken thigh (C).



of Mb from the HPLC system was  $93.1 \pm 1.300\%$ , which meant that little heme pigment was retained in the HPLC column. The minimum detectable level for Mb by the SE method was  $0.001 \mu\text{g}/\mu\text{l}$ . This procedure required about 30 minutes to finish one sample determination starting with preparation of sample extracts.

The high performance liquid chromatogram of standard bovine cytochrome c, Mb, and Hb with hydrophobic interaction chromatography (HIC) showed elution of cytochrome c, Mb and Hb at 4.35, 11.40 and 15.70 minutes, respectively (Figure 3.2A). The two pigment peaks of bovine samples had the same shapes and retention times as Mb and Hb standards, respectively (Figure 3.2B). The absorbance ratio for a pure compound at any two wavelengths should be constant (Kakuda et al., 1981). The absorbance ratio of 280:320 nm for the peak corresponding to Mb for longissimus dorsi and psoas major were 1.24 and 1.26, respectively, which almost equaled the ratio of the standard Mb solution (1.27). The absorbance ratio for the peak corresponding to Hb for the same samples were 1.38 and 1.40 with the same wavelengths, respectively, which almost equaled the ratio of the standard Hb solution (1.40). These results strongly supported the labeling of the two peaks in the bovine samples as the respective Mb and Hb peaks.

The recoveries of Hb and Mb from the HPLC system were  $90.4 \pm 4.370\%$  and  $97.5 \pm 0.935\%$ , respectively, which

suggested that little heme pigment was retained in the HPLC column. The minimum detectable levels for Hb and Mb were  $0.186 \mu\text{g}/\mu\text{l}$  and  $0.085 \mu\text{g}/\mu\text{l}$ , respectively, indicating that the hydrophobic interaction column had a higher sensitivity to Mb than to Hb. It took about 35 minutes to finish one sample determination starting from the preparation of the sample.

The size exclusion column should have separated Hb and Mb if their sizes were different. Because the same procedures were used in the preparation of the meat extract for the separations by SE and HIC, and HIC could separate Mb and Hb in the extract, it was unlikely that Hb would dissociate during the preparation of the extract. Hb is composed of four polypeptide chains held together by noncovalent attractions. Noncovalent attractions, such as hydrogen bonds and Van Der Waal's forces, are much weaker than the covalent forces (Stryer, 1988). An explanation for Mb and Hb standards eluting as peaks at 11.30 minutes separately or in combination is that the noncovalent bonds holding the four polypeptide chains in Hb might be dissociated by the column separation forces associated with the SE column.

Mb was used as a standard to quantify the content of total pigments in muscle since the percentage of Mb in well-bled muscle tissue was much higher than for Hb (Fennema, 1985; Judge et al., 1989). The different retention times

between cytochrome c (14.06 min.) and heme pigments (11.30 min.) indicated that the small amount of cytochrome c in muscle sample was not included in the total pigment content by this HPLC size exclusive method. Because the objective of this research was to determine heme pigments in muscle, no efforts were made to identify peaks other than those discussed above in the HPLC chromatograms (Figures 3.1B and 3.1C).

The same procedure was used to prepare the sample extracts for SE and HIC. The Mb and Hb in bovine muscle were completely separated by the hydrophobic interaction column (Figure 3.2B). The hydrophase HP-butyl column contains a 100 x 7.8 mm bed of 10  $\mu$ m HP-butyl hydrophilic polymer containing n-butyl groups covalently bound to a hydrophilic matrix (Interaction, 1989). The n-butyl groups exhibit hydrophobic interaction characteristics. Hb is composed of four heme groups and four polypeptide chains, while there is only one heme group and a polypeptide chain for Mb (Fennema, 1985). Sample components which are loaded on the column are eluted according to their degree of hydrophobic interaction with the packing contained in the column and their solubility in the salt buffers. Proteins with weaker hydrophobic attractions elute sooner than proteins with stronger hydrophobicity (Nelson, 1989). Therefore, the Hb was shown to contain more hydrophobic groups than Mb since it eluted from the column later than Mb (Figures 3.2A and 3.2B). The Mb or Hb peaks

from chicken samples were not noticeable (Figure 3.2C), which meant that Mb and Hb in chicken could not be separated by the HIC column. The possible explanation for the result was that the concentration of the heme pigments in chicken muscle was too low to be detected by the HIC column.

#### **Quantitation of total pigments, Mb and Hb**

Standard curves of total pigments, Mb, and Hb were the regression lines of the peak areas of the concentrations of the standards, which were used to calculate the corresponding pigment concentrations in the samples. The standard curves used in the experiment are given in Appendix Figures 1, 2, and 3.

The results of statistical analyses for the concentration of total pigments in beef and chicken muscles are in Appendix Table 1. The results suggested that the concentration of total pigments differed among the cattle ( $p < 0.05$ ). Interactions existed ( $p < 0.05$ ) between cattle and type of muscle (longissimus dorsi and psoas major). There were no interactions ( $p > 0.05$ ) between the analytical method and type of muscle.

The concentration of total pigments also differed among different chickens ( $p < 0.05$ ). Interactions existed ( $p < 0.05$ ) between individual chicken and the type of muscle (drumstick and thigh). There were no interactions between the method and type of muscle in determining the total pigment contents in chicken.

The interactions ( $p < 0.05$ ) between cattle or chicken and type of muscle (bovine longissimus and psoas major, or chicken thigh and drumstick) (Appendix Table 1) suggested that pigment contents in the two kinds of muscles varied with individual cattle or chicken. There were no interactions ( $p > 0.05$ ) between analytical method and type of muscle, indicating that the SE and spectrophotometric methods were consistent in determining the total pigment contents in beef and chicken.

The least-squares means (LSM) of total pigment contents in bovine longissimus dorsi and psoas major and in chicken drumstick and thigh determined by SE, HIC, and spectrophotometric methods are summarized in Table 3.1. For bovine muscles, the SE method produced the highest mean value of total pigments, while HIC method gave the lowest mean value of total pigments. The SE method also provided the lowest standard deviation of the methods tested (Table 3.1).

The concentrations of total pigments, Mb and Hb in bovine muscle obtained by the methods in this study were in agreement with literature values. The ranges of total pigment, Mb and Hb concentrations previously reported were 2.8-5.0 mg/g, 2.0-5.0 mg/g and 0.62-0.68 mg/g in bovine muscle, respectively (Fleming et al., 1960; Hunt and Hedrick, 1977; Águllo et al., 1990; Oellingrath et al., 1990). The total pigment concentrations in chicken dark muscles were similar to the 0.25-0.27 mg/g reported by Ball (1986), but

slightly lower than the results of Saffle (1973) (0.33-0.50 mg/g dark meat of eight week old chicken). The genetic changes in broiler chickens over the last 20 years should be taken into consideration in interpreting the similarity and differences among the results of various studies.

**Table 3.1 Least-squares means of total pigment contents<sup>a</sup> with different analytical techniques.**

Muscle type	Analytical method		
	HIC <sup>b</sup>	SE <sup>c</sup>	Spectrophotometry
Bovine longissimus <sup>d</sup>	4.26 <sup>g</sup> (0.92)	4.96 <sup>i</sup> (0.42)	4.52 <sup>gh</sup> (0.65)
Bovine psoas <sup>d</sup>	4.52 <sup>gh</sup> (0.77)	4.72 <sup>hi</sup> (0.39)	4.50 <sup>gh</sup> (0.43)
Chicken drumstick <sup>e</sup>	ND <sup>f</sup>	0.12 <sup>j</sup> (0.01)	0.11 <sup>j</sup> (0.02)
Chicken thigh <sup>e</sup>	ND <sup>f</sup>	0.21 <sup>k</sup> (0.03)	0.19 <sup>k</sup> (0.04)

<sup>a</sup>Mean (mg/g meat) and standard deviation in parentheses for total pigment concentration.

<sup>b</sup>Hydrophobic interaction chromatography with individual Mb and Hb contents summed for total pigment determination.

<sup>c</sup>Size exclusion chromatography with Mb and Hb eluting as a single peak.

<sup>d</sup>Data from 5 cattle with standard error of mean = 0.14.

<sup>e</sup>Data from 3 broilers with standard error of mean = 0.01.

<sup>f</sup>ND=not detectable

<sup>g,h,i,j,k</sup>Means not bearing a common superscript letter are different (P>0.05).

The lower standard deviations (Table 3.1) of the SE method indicated that it was more consistent in measuring total pigments than either the spectrophotometric method or the HIC method. The SE method also provided the greatest

sensitivity in measuring total pigments since it had the lowest detectable level of total pigments (the lowest amount of total pigments determined by spectrophotometric analysis was  $0.037 \mu\text{g}/\mu\text{l}$ ). Therefore, the SE method was more favorable for the determination of total pigments especially when used for those species having low Mb content such as poultry.

Analysis of variance (Appendix Table 2) showed that differences ( $p < 0.05$ ) occurred for Mb or Hb concentrations among cattle, which suggested that large variations of Mb and Hb contents existed even among animals of the same species of similar breedtypes and production backgrounds. Interactions ( $p < 0.05$ ) existed between cattle and type of muscle, indicating that Mb and Hb contents in the two kinds of muscles varied with individual cattle. The least-squares means of Mb and Hb in bovine longissimus and psoas major determined by HIC are summarized in Table 3.2.

The separation of Mb and Hb can enhance the accuracy of evaluating muscle color or bleeding method since Mb is primarily a muscle pigment while Hb is a pigment protein in the blood. The Hb content in muscle is determined by the extent of bleeding of the carcass during slaughter (Oellingrath et al., 1990). A molecule of Mb contains only 1 heme iron while a molecule of Hb contains 4 heme irons. With the information on Hb and Mb contents in muscles, the heme iron content in raw meat can be accurately calculated.

**Table 3.2 Least-squares means of Mb and Hb contents<sup>a</sup> in bovine muscle with HIC.**

Cattle	Myoglobin <sup>b</sup>		Hemoglobin <sup>c</sup>	
	Longissimus	Psoas	Longissimus	Psoas
1	2.66 <sup>d</sup> (0.19)	2.48 <sup>d</sup> (0.19)	0.64 <sup>h</sup> (0.01)	0.75 <sup>hi</sup> (0.06)
2	2.47 <sup>d</sup> (0.10)	3.93 <sup>efg</sup> (0.80)	1.04 <sup>ij</sup> (0.13)	0.82 <sup>hij</sup> (0.28)
3	4.27 <sup>fg</sup> (0.05)	3.68 <sup>ef</sup> (0.12)	0.80 <sup>hi</sup> (0.05)	0.83 <sup>hij</sup> (0.04)
4	3.16 <sup>dc</sup> (0.58)	3.63 <sup>ef</sup> (0.06)	0.91 <sup>hij</sup> (0.07)	1.22 <sup>j</sup> (0.05)
5	4.65 <sup>g</sup> (0.08)	4.31 <sup>fg</sup> (0.37)	0.70 <sup>hi</sup> (0.32)	0.97 <sup>hij</sup> (0.33)

<sup>a</sup>Each value (mg/g meat) is the least-square means of three determinations with standard deviation in parenthesis.

<sup>b</sup>The standard error of mean for Mb = 0.25.

<sup>c</sup>The standard error of mean for Hb = 0.13.

<sup>defghij</sup>Means not bearing a common superscript letter are different (P>0.05).

The heme iron content is an important index in estimating the amount of absorbable iron in meat, because heme iron has a much high bioavailability than nonheme iron (Monsen et al., 1978; Mahoney, 1986), and in estimations on the lipid stability as Mb is a more effective catalyst of lipid oxidation than Hb (Sliberstein and Lillard, 1978). In addition, the determination of total pigments by the HIC method was in agreement with spectrophotometric techniques, but was less sensitive than the other two methods.

The Hydrophase HP-butyl column contained a HP-butyl hydrophilic polymer to which n-butyl groups were covalently



bound (Interaction, 1989). After prolonged use of the column, the sensitivity to Hb was reduced, probably due to coating of its polymer surface by fats and lipids from the meat samples. Therefore, the column needed to be regenerated by washing with dilute HCl and NaOH to remove fat and lipid at a frequency of every 5 sample injections. Águllo et al. (1990) reported that trichloroethylene could remove lipid and other proteins from the aqueous extract. However, it was found in this study that there was no significant improvement in the sensitivity of the column to Hb by using trichloroethylene. Methods to remove fat and lipid from samples prior to SE and HIC analyses need to be further studied.

The SE method had advantages of high sensitivity and measurement consistency and, therefore, is recommended for determination of total pigments in muscles, especially for those species with low Mb content such as poultry. The HIC method was convenient in separating Mb and Hb in beef muscle but less sensitive than SE or spectrophotometric techniques.

## **CHAPTER IV**

### **EXPERIMENT TWO: EFFECT OF HEAT ON THE DISTRIBUTION OF IRON IN BEEF AND CHICKEN MUSCLES**

## INTRODUCTION

Iron in muscle is present in several different compounds, including low molecular weight molecules, heme compounds such as myoglobin (Mb), hemoglobin (Hb), and hematin, and storage complexes of ferritin and hemosiderin (Torrance et al., 1968; Hazell, 1982). Most of the iron in meat is associated with muscle pigment Mb; a lesser amount is found in blood pigment Hb (Love, 1987). Meat is a rich source of highly available iron, since heme iron has been reported to have a much higher bioavailability than other forms of iron (Monsen et al., 1978; Mahoney, 1986). To make estimates of the total available iron in a meal, it is important to know the content of different forms of iron in the foods. Iron is also the major catalyst for the development of oxidative rancidity in meat (Love, 1987), but the catalytic abilities of various forms of iron for lipid oxidation are different. Research has indicated that the major catalyst of lipid oxidation might be heme iron in raw red meats (Younathan and Watts, 1959; Tappel, 1962; Rhee, 1988) and nonheme iron in cooked meats (Sato and Hegarty, 1971; Love and Pearson, 1974; Igene et al., 1979).

Several studies have shown that heating decreased the heme iron content and increased the nonheme iron content in meat or meat extracts (Igene et al., 1979; Schricker et al., 1982; Chen et al., 1984). The change of the distribution of iron in various fractions in meats would affect the

bioavailability of iron and both the degree and rate of lipid oxidation in cooked meat. However, there are few studies concerning the effect of heat on the amounts of iron in various meat fractions. Based on the assumption that only heme iron is insoluble in the acidic conditions, most previous investigations have focused on determinations of the contents of heme and nonheme irons after solubilization and precipitation with trichloroacetic acid (TCA) (Igene et al., 1979; Schricker et al., 1982). Further information on the effect of heat on the distribution of the different iron fractions in meat could enhance the understanding of the roles of various forms of iron in lipid oxidation. This information could also be used to more accurately estimate the total available iron in meals containing meat.

The objective of this study was to determine the effect of increased temperatures on the distribution of different fractions of iron in beef and chicken muscles.

## MATERIALS AND METHODS

### Samples

Approximately 250-g of longissimus dorsi muscle (pH 5.6) were obtained from each of five beef carcasses at the Louisiana State University Agricultural Center Meat Laboratory. All visible fat and connective tissues were trimmed and cut into small pieces (about 1.25 cm square each) with a knife. Samples weighing 165-g were selected randomly from the trimmed and cut meat from each of the five cattle, combined, and mixed, and then ground twice through a 4.7 mm stainless steel plate. The ground bovine muscle was randomly separated into 80-g batches which were vacuum-packaged in vinyl bags and stored at  $-18^{\circ}\text{C}$  for less than 15 days. Thighs from five refrigerated chicken (about 1.8 kg each) were purchased from a local supermarket in Baton Rouge, prepared similarly to the procedure for beef, and stored at  $-18^{\circ}\text{C}$ .

The ground bovine longissimus samples had a moisture content of  $71.23 \pm 0.15\%$  and a crude fat content of  $4.05 \pm 0.08\%$  as determined by rapid microwave procedures (AVP80 and Automatic Extraction System, CEM Corporation, Matthews, NC). The moisture and crude fat contents of the ground chicken samples were  $76.15 \pm 0.23\%$  and  $3.26 \pm 0.09\%$ , respectively.

### Method of heating

Frozen vacuum-packaged samples were thawed at room temperature ( $27^{\circ}\text{C}$ ) for two hours. Each 80-g thawed sample of ground muscle was mixed with 80 ml deionized water in a

blender (Waring, Hartford, CT) at low speed for 30 seconds to obtain a semi-liquid meat slurry for more uniform heat transfer. The meat slurries were transferred to 250 ml Erlenmeyer flasks with well-fitting stoppers and placed inside 500-ml beakers in a steam water bath at 101.6°C (Precision Scientific Co., Chicago, IL). The temperature of the water in the covered water bath was higher than 100°C because of the generation of some steam in the container. Four flasks with samples were placed in the steam water bath simultaneously. The flasks were randomly assigned for removal from the water bath when internal temperatures of 55°C, 70°C, 85°C, or 100°C were reached. These temperatures were selected because previous studies suggested that at 55°C, Mb would not be denatured (Saffle, 1973) while at 70°C, lipid oxidation still increased (Keller and Kinsella, 1973; Pearson et al., 1977). In meat cooked above 80°C, lipid oxidation would decrease (Pearson et al., 1977); and 100°C is the boiling temperature. Consistent intervals between the heat temperatures were selected due to the practical concern in data analysis. A thermocouple to detect temperature changes was placed in the center of the sample in each flask through a perforated hole in the stopper. The thermocouples were linked to the Digital Data Acquisition System (MackMac 1240-Touch Display, GreenSpring Computers, Menlo Park, CA) which used the Thermo VI program to monitor temperature changes. The heating rate curves of beef and chicken

slurries in the water bath are shown in Appendix Figure 4. The time periods required to reach the internal temperatures of 55°C, 70°C, 85°C, and 100°C in the beef slurries were 6.2, 12.05, 21.8, and 47.9 minutes, respectively. The time periods needed to reach the internal temperatures of 55°C, 70°C, 85°C, and 100°C in the chicken slurries were 6.4, 12.05, 21.8, and 48.7 minutes, respectively. Flasks were cooled to room temperature (27°C) in ice water after removal from the water bath. The cooked samples were then transferred to 500-ml blending jars and mixed in a blender (Waring, Hartford, CT) at low speed for 30 seconds to obtain a homogenous meat slurry. The experiment had two replications, and four samples of beef or chicken, 2 samples from each replication, were selected at each heating temperature for iron determination. Raw beef or chicken samples were used as controls which were kept at room temperature (27°C).

#### **Determination of total iron**

The methods used to determine various forms of iron in meat samples are outlined in Appendix Figure 5. Meat slurries were digested with a mixture of nitric acid and perchloric acid (7:1 by volume) following procedures of Guzman (1987) (Appendix Method 1). Total iron contents were determined on wet ashed samples by atomic absorption spectrophotometry (AAS) (Model 3030B, Perkin Elmer Corp., Norwalk, CT) at a wavelength of 248.3 nm and a slit width of 0.2 nm with an air-acetylene oxidizing flame (Guzman, 1987).

The amount of iron in different fractions of beef or chicken muscle were expressed as  $\mu\text{g}$  iron/g of muscle.

#### **Determination of iron in water-soluble and insoluble fractions**

About 20 grams of meat slurry were weighed into a 500-ml blending jar and homogenized in a blender (Waring, Hartford, CT) at low speed for 2 minutes. The homogenates were transferred to 50-ml centrifuge tubes and centrifuged at 3000 x g for 20 minutes for beef samples and 40 minutes for chicken samples. A longer centrifugation time was necessary for chicken extracts to have the same clarity as beef extracts. The top solution was decanted into a 100-ml beaker. The meat residues were re-homogenized with 10 ml of deionized water, re-centrifuged, and the supernatant was decanted into the 100-ml beaker until there was a total of four extractions. All supernatants were filtered (No. 3, Whatman International Ltd., Maidstone, England), wet ashed with the mixture of nitric acid and perchloric acid, and the iron contents of water soluble fractions were determined by AAS as described previously. The meat residues after decanting were also wet-ashed and the iron contents of water-insoluble fractions were determined by AAS.

#### **Determination of diffusate iron**

Supernatants of meat slurries after water solubilization as described in the previous section were sampled. Ten-ml aliquots were dialyzed against 30 ml of deionized water at



4°C for 48 hours using 4.5 cm standard dialysis tubing (Fisher Scientific, Pittsburgh, PA). The molecular weight cutoff of the dialysis tubing was 12,000 daltons, i.e. compounds with molecular weight less than 12,000 daltons permeated the dialysis tubing. After a 48-hr dialysis period, the diffusate fraction (i.e. the dialyzable fraction or the low molecular weight fraction) was wet-ashed with the mixture of nitric acid and perchloric acid. The iron contents in the diffusate fractions were then determined by AAS.

#### **Determination of hematin iron content**

About 10 grams of meat slurry were weighed into a 500-ml blending jar and homogenized with 15 ml of acetone in a blender (Waring, Hartford, CT) at low speed for 2 minutes to extract hematin (free heme) at neutral pH. The homogenates were centrifuged at 3000 x g for 20 minutes in covered centrifuge tubes. The acetone extract was decanted into a 50-ml beaker, acidified to pH 2.5 with 2.5 ml of 1 N HCl to intensify the red color of heme, and filtered (No.3 filter paper, Whatman International Ltd., Maidstone, England). The hematin content in the acidified acetone extract was then determined by measuring the optical density at 540 nm by a spectrophotometer (Model U-2000 UV/Vis Spectrophotometer, Danbury, CT) (Lewis, 1954; Ladikos and Wedzicha, 1988). Hemin chloride (Sigma Chemical Co., St. Louis, MO) was used as a standard. The concentrations of hemin chloride in

acidified acetone (pH 2.5) used to make the standard curve were  $1.175 \times 10^{-3}$ ,  $2.35 \times 10^{-3}$ ,  $3.525 \times 10^{-3}$ , and  $4.7 \times 10^{-3}$   $\mu\text{g}/\mu\text{l}$ . Hematin concentration in samples was calculated from the standard curve of hemin chloride (Appendix Figure 6), and the iron content in hematin in the samples was calculated by the following equation:

$$\text{Iron content } (\mu\text{g/g}) = \text{Hematin content } (\mu\text{g/g}) \times \text{AW} / \text{MW}$$

where AW was the atomic weight of iron (55.84), and MW was the molecular weight of hemin chloride (652).

#### **Determination of iron content in total heme fraction**

About 10 grams of meat slurry were weighed into a 500-ml blending jar and acidified to pH 2.5 with addition of 2.5 ml of 1 N HCl before homogenization with 37.5 ml of acetone for 2 minutes. The homogenates were centrifuged at  $3000 \times g$  for 20 minutes in covered centrifuge tubes. The acetone extract was then filtered through No. 3 filter paper (Whatman International Ltd., Maidstone, England). The absorbance of the acetone extract was read spectrophotometrically at 540 nm. Hemin chloride (Sigma Chemical Co., St. Louis, MO) was used as a standard. The concentrations of hemin chloride in acidified acetone (pH 2.5) used to make the standard curve were  $4.7 \times 10^{-3}$ ,  $9.4 \times 10^{-3}$ ,  $1.41 \times 10^{-2}$ ,  $1.88 \times 10^{-2}$ , and  $2.115 \times 10^{-2}$   $\mu\text{g}/\mu\text{l}$ . Total heme concentration in samples was calculated from the standard curve of hemin chloride (Appendix Figure 7). Iron content in the total heme fraction

was calculated by the same equation as described previously for the hematin iron determination.

#### **Determination of iron content in ferritin fraction**

Ferritin in meat slurries was separated from other water soluble proteins by gel filtration chromatographic techniques (Hazell, 1982). Supernatants of meat slurry prepared as the water-soluble fractions were used as samples for the separation. A gel with a fractionation range from 800,000 to 12,000 daltons (Ultrogel AcA 34 gel, Spectrum, Los Angeles, CA) was packed in a column (100 cm long and 2.5 cm diameter) for the separation.

In order to determine the retention time of ferritin fraction from the column, an identification experiment was conducted. Ferritin from horse spleen (Sigma Chemical Co., St Louis, MO) was used as the standard to identify the ferritin peak of the samples, because ferritin from bovine and chicken were not available commercially. A 10-ml ferritin standard solution (0.2 mg/ml) or sample supernatant was loaded on the column and eluted with 0.2 M potassium orthophosphate buffer (pH 6.8) at a flow rate of 0.42 ml/minute (Hazell, 1982). At 12 hours after loading of the sample, fractions were collected at 2-minute intervals. The absorption of collected fractions were measured at 540 nm in a spectrophotometer to determine the existence of ferritin in each fraction. The chromatograms of the aqueous extracts of beef and chicken muscles on the gel filtration column are

given in Appendix Figure 8. The ferritin standard was eluted out from the column between the 9th and 16th fraction number (Appendix Figure 8). The sample peak was identified as ferritin fraction if it had the same retention time as the standard ferritin.

Because the retention time of ferritin was identified, a 10-minute interval was decided to be appropriate in the collection of fractions from samples for the ferritin iron determination. The fractions containing ferritin of the samples (two fractions collected from 10 to 20 minutes, and 20 to 30 minutes) were pooled and wet-ashed with the mixture of nitric acid and perchloric acid. Iron contents in the ferritin fractions were then determined by AAS.

#### **Statistical analysis**

The experimental design was a randomized block design (RBD) with replication as block and temperature as treatment. Analyses of variance (ANOVA) were used for data analysis (SAS, 1985b). Least-squares means (LSM) were compared using t-tests when analyses of variance indicated temperature effects ( $p < 0.05$ ).

## RESULTS AND DISCUSSION

Analyses of variance (Appendix Tables 3 and 4) indicated that temperature affected ( $p < 0.05$ ) the iron content in water-soluble, dialyzable, hematin, total heme and ferritin fractions in both beef and chicken muscle. The iron content in water-insoluble fractions of beef were different ( $p < 0.05$ ) at various temperatures. However, the amount of iron in the water-insoluble fractions of chicken thigh did not change ( $p > 0.05$ ) as temperature increased.

The least-squares means (LSM) of iron contents in total iron, water soluble, water insoluble, dialyzable, hematin, total heme, and ferritin fractions at different temperatures in bovine longissimus and chicken thigh are listed in Tables 4.1 and 4.2, respectively. The total iron in bovine longissimus and chicken thigh muscles did not change ( $p > 0.05$ ) with the increase in temperature. The total iron content in beef longissimus muscle was about 10  $\mu\text{g/g}$  greater than the total iron content in chicken thigh.

The concentration of total iron in the experiment was slightly higher for bovine longissimus and was higher for chicken thigh than reported in previous studies. The range of total iron in bovine longissimus reported in the literature was 17.5-24.5  $\mu\text{g/g}$  (Schricker et al., 1982; Hazell, 1982; Love, 1988), while that in chicken thigh was 8.8-13.2  $\mu\text{g/g}$  (Hazell, 1982; Love, 1988). Several factors might contribute to the higher iron content of the muscle

samples in the current study, including incomplete bleeding during slaughtering, lower fat content, and different breedtypes.

**Table 4.1** Iron contents<sup>a</sup> ( $\mu\text{g}$  Fe/g sample) in various fractions of bovine longissimus muscle at different temperatures.

Iron Type	Temperature					SEM <sup>c</sup>
	Control <sup>b</sup>	55°C	70°C	85°C	100°C	
Total Fe	24.56	24.88	24.76	24.55	24.80	0.99
H <sub>2</sub> O Soluble Fe	16.93 <sup>c</sup>	15.20 <sup>c</sup>	8.18 <sup>f</sup>	6.61 <sup>f</sup>	5.65 <sup>f</sup>	0.99
H <sub>2</sub> O Insoluble Fe	8.11 <sup>c</sup>	9.41 <sup>c</sup>	16.45 <sup>f</sup>	17.29 <sup>f</sup>	18.36 <sup>f</sup>	1.29
Diffusate Fe	1.74 <sup>c</sup>	1.80 <sup>c</sup>	2.16 <sup>f</sup>	2.76 <sup>g</sup>	2.77 <sup>g</sup>	0.09
Fe in Hematin	0.24 <sup>c</sup>	0.28 <sup>cf</sup>	0.28 <sup>cf</sup>	0.29 <sup>f</sup>	0.35 <sup>g</sup>	0.02
Fe in Total Heme	14.46 <sup>c</sup>	14.08 <sup>c</sup>	13.08 <sup>f</sup>	11.82 <sup>g</sup>	11.74 <sup>g</sup>	0.14
Fe in Ferritin	0.32 <sup>c</sup>	0.25 <sup>c</sup>	0.13 <sup>f</sup>	ND <sup>d</sup>	ND <sup>d</sup>	0.03

<sup>a</sup>Each value is the least-squares means of four measurements.

<sup>b</sup>Raw meat was used as control samples which was kept at the room temperature (27°C).

<sup>c</sup>SEM=standard error of the least-squares means.

<sup>d</sup>ND=not detectable.

<sup>cf</sup>Means in the same row not bearing a common superscript letter are different ( $p < 0.05$ ).

The sum of iron in water soluble and insoluble fractions for both beef and chicken was similar to the total iron value at each temperature (difference ranged from 0.53% to 3.2% for beef and from 1.44% to -3.75% for chicken). Raw beef

contained a much higher percentage of soluble iron (68.9% of total iron) and a lower percentage of insoluble iron (33% of total iron) than raw chicken, which contained 37.9% of soluble iron and 63.4% of insoluble iron. The main iron-containing proteins in the water extracts from meat are the hemoproteins, Mb and Hb (Hunt and Hedrick, 1977; Hazell, 1982; Love, 1988). The higher percentage of water soluble

**Table 4.2** Iron contents<sup>a</sup> ( $\mu\text{g}$  Fe/g sample) in various fractions of chicken thigh at different temperatures.

Iron Type	Temperature					SEM <sup>c</sup>
	Control <sup>b</sup>	55°C	70°C	85°C	100°C	
Total Fe	14.54	14.49	14.54	14.61	14.93	0.69
H <sub>2</sub> O Soluble Fe	5.51 <sup>c</sup>	4.87 <sup>c</sup>	2.93 <sup>f</sup>	2.83 <sup>f</sup>	2.82 <sup>f</sup>	0.23
H <sub>2</sub> O Insoluble Fe	9.22	10.12	11.40	12.07	12.67	0.98
Diffusate Fe	1.01 <sup>c</sup>	1.05 <sup>c</sup>	1.22 <sup>cf</sup>	1.46 <sup>f</sup>	1.46 <sup>f</sup>	0.09
Fe in Hematin	0.15 <sup>c</sup>	0.16 <sup>c</sup>	0.18 <sup>c</sup>	0.20 <sup>c</sup>	0.31 <sup>f</sup>	0.02
Fe in Total Heme	2.44 <sup>c</sup>	2.40 <sup>c</sup>	2.19 <sup>g</sup>	1.98 <sup>h</sup>	1.87 <sup>h</sup>	0.05
Fe in Ferritin	0.50 <sup>c</sup>	0.40 <sup>f</sup>	0.23 <sup>g</sup>	ND <sup>d</sup>	ND <sup>d</sup>	0.02

<sup>a</sup>Each value is the least-square means of four measurements.

<sup>b</sup>Control samples were maintained at room temperature (27°C).

<sup>c</sup>SEM=standard error of the least-squares means.

<sup>d</sup>ND=not detectable.

<sup>e,f,g,h</sup>Means in the same row not bearing a common superscript letter are different ( $p < 0.05$ ).

iron in the raw bovine longissimus muscle is because beef had a higher pigment content than chicken thigh (Table 3.1).

Iron contents in various fractions of beef and chicken muscle followed different patterns of change as temperature increased. Figure 4.1 and Figure 4.2 show the patterns of iron content changes in water soluble and insoluble fractions of beef and chicken muscle, respectively, as the temperature increased. Iron content in water soluble fractions decreased and insoluble fractions increased in beef muscle with increased temperature. Compared to the corresponding iron content in raw beef, iron content in water soluble and insoluble fractions decreased 66.6% and increased 126% at 100°C, respectively. Heating to 70°C had the greatest effect on the iron content in the water soluble and insoluble fractions of bovine and chicken muscles. The results suggested that most hemoproteins might be denatured between the temperatures of 55°C and 70°C.

The iron contents in water soluble and insoluble fractions of chicken muscle changed less than the ones in bovine muscle with increased temperature. This finding might be explained by chicken having a high percentage of insoluble iron than soluble iron initially while beef had a higher percentage of soluble iron than insoluble iron at room temperature (Tables 4.1 and 4.2). The small increase for insoluble iron in chicken due to the denaturation of the hemoproteins by heat did not result in a significant change



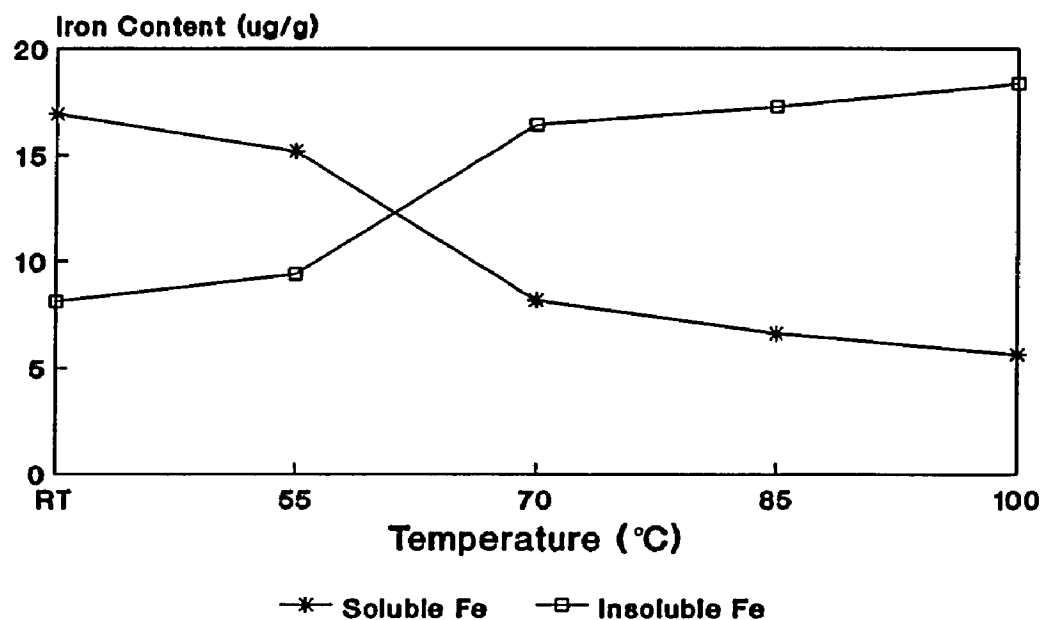


Figure 4.1 Effect of temperature on the iron contents in the water soluble and insoluble fractions in bovine longissimus muscle.

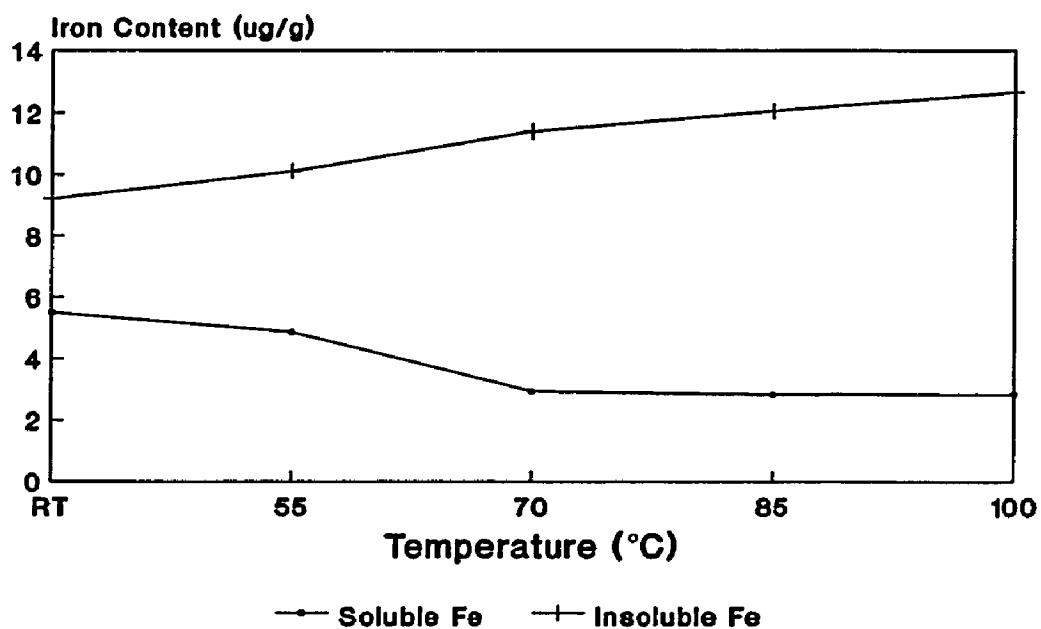


Figure 4.2 Effect of temperature on the iron contents in the water soluble and insoluble fractions in chicken thigh.

in the insoluble iron content at different temperatures. The precise nature of the insoluble iron in meat was unknown. Some of it might be hemosiderin, an iron storage compound which has been detected in muscle and which can occur in large concentrations in the liver (Martinez-Torres et al., 1976; Torrance et al., 1968).

The changes in diffusate iron for both beef and chicken were similar (Figure 4.3). The concentration of diffusate iron increased ( $p < 0.05$ ) as the temperature increased to 85°C. There was no further increase in diffusate iron as temperature increased from 85°C to 100°C. The increased diffusate iron might come from the breakdown of heme ring or/and the release of ferritin, to be discussed later.

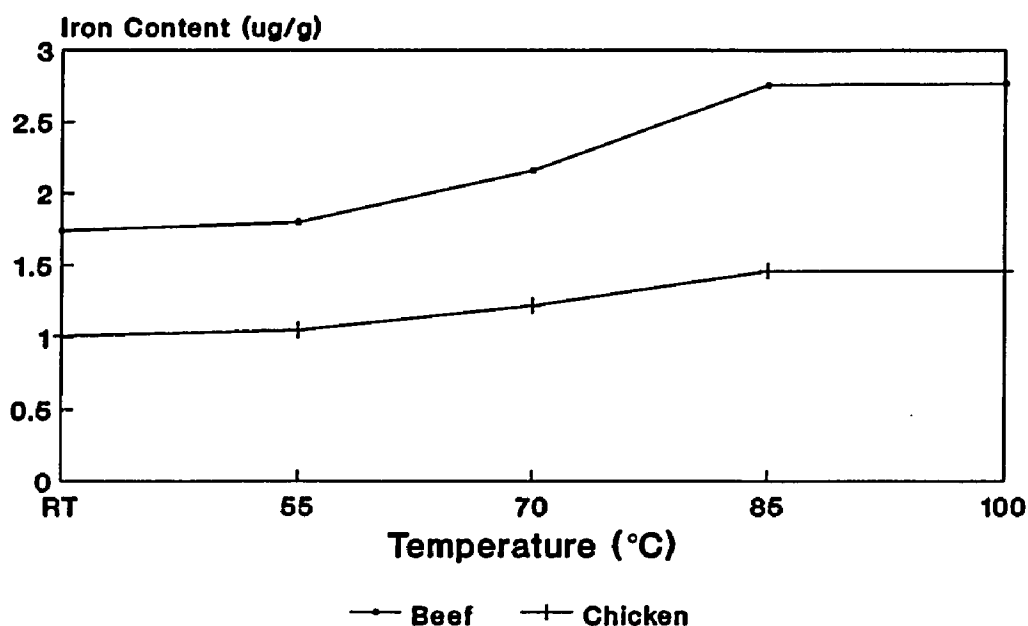
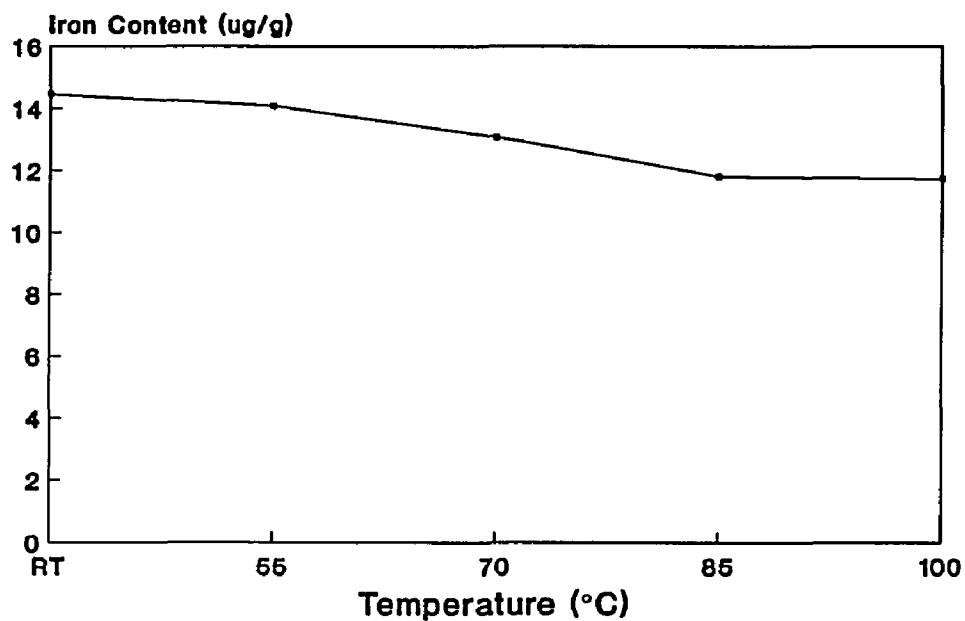


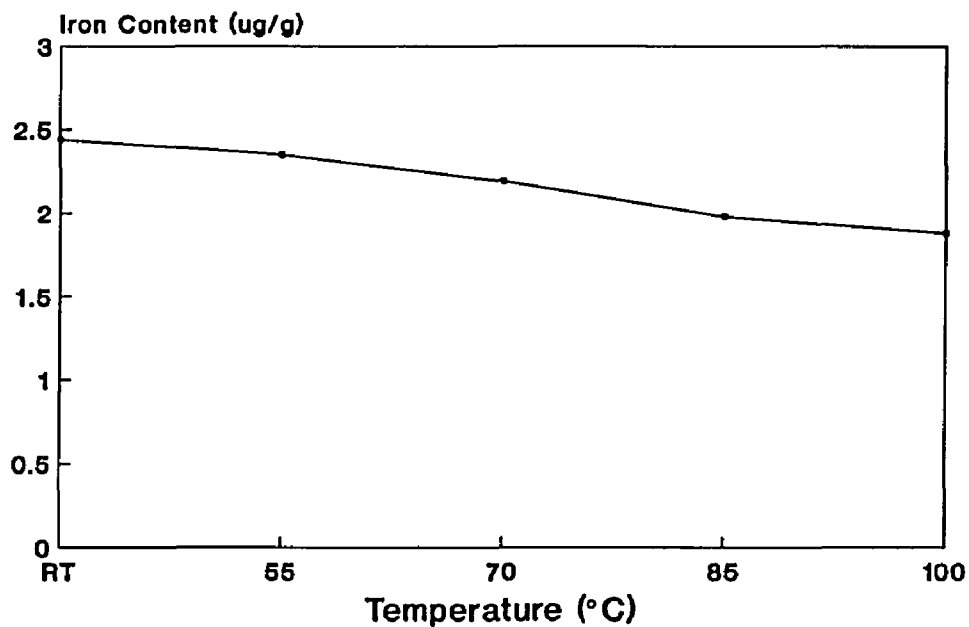
Figure 4.3 Effect of temperature on the contents of diffusate iron in beef longissimus and chicken thigh muscles.

Heme compounds in meat include hematin, Hb, Mb, cytochrome c, and several heme-containing enzymes such as catalase and peroxidase (Apte and Morrissey, 1987a; Stryer, 1988). Hematin is a heme group having two coordinate water molecules or hydroxyl groups (Ladikos and Wedzicha, 1988). Hematin is insoluble in water but soluble in acetone. Hb and Mb, which are soluble proteins, are composed of the heme moiety, an iron atom surrounded by a porphyrin ring, and polypeptide chains of amino acids (Dickerson and Geis, 1983; Ladikos and Wedzicha, 1988). The heme moiety in hemoproteins is not a fixed prosthetic group. Reversible dissociation into heme and apoproteins may be achieved (Rossi Fanelli et al., 1958). However, in both hemoglobin and myoglobin, the affinity of the heme for the protein at neutral pH is very high (Fronticelli and Bucci, 1963), with the heme-globin equilibrium constant estimated to be of the order of  $10^{12}$  -  $10^{15}$  M (Gibson and Antonini, 1963). Dissociation of the heme is increased considerably at acid pH (Lewis, 1954). If the pH of a hemoprotein solution is less than 3, the characteristic linkage of the prosthetic group with the protein is ruptured and the protein is denatured (Lewis, 1954; Ladikos and Wedzicha, 1988). The cleaved heme dissolves in acetone and has a well defined spectrum band at 540 nm, while the free protein precipitates (Lewis, 1954). These properties of heme were used to determine the hematin and total heme contents in muscle in the present experiment.

Although hemoproteins in meat were denatured and became insoluble in water at high temperature, most of their heme moiety was not destroyed even at temperature of 100°C (Tables 4.1 and 4.2). The change in total heme content in beef and chicken were similar (Figures 4.4 and 4.5). The concentrations of total heme iron in beef and chicken at 100°C only decreased 22.6% and 23.3%, respectively, compared with the corresponding total heme iron content in raw samples. Most of the decrease of heme iron in beef and chicken occurred at temperatures from 55°C to 85°C. The greater reduction of heme iron in beef and chicken at temperatures from 55°C to 85°C was consistent with the results reported by Chen et al. (1984) that the optimal temperature for the release of iron from heme was between 63°C and 70°C. Schricker et al. (1982) indicated that the decrease of heme iron in meats by heat was due to the oxidative cleavage of the porphyrin ring of heme. In the present study, an increase in diffusate iron should be reflected by oxidative cleavage of the heme porphyrin ring because the molecule weight of the heme is 617.5 daltons. However, the amount of increased diffusate iron was smaller than the amount of decreased heme iron at each temperature level in both beef and chicken samples (Tables 4.1 and 4.2). These results suggested that oxidative cleavage of the porphyrin ring was not the only means by which heme iron decreased, but other changes in the heme might occur during heating to cause



**Figure 4.4** Effect of temperature on the content of total heme iron in beef longissimus muscle.



**Figure 4.5** Effect of temperature on the content of total heme iron in chicken thigh.

insolubility in acidified acetone and thus decreased heme iron. temperatures and became insoluble in acidified acetone which caused the decrease of heme iron.

When the heating temperature reached 85°C, hematin content in beef samples began to show a slight ( $p < 0.05$ ) increase compared with the control group. Hematin content was increased more ( $p < 0.05$ ) in both beef and chicken samples when the heating temperature reached 100°C (Figure 4.6). The slight increase in the hematin content in beef and chicken with the increased temperatures meant that most hemoproteins did not release their heme moiety during the denaturation. Most heme was still associated with globin in cooked meat. As temperature increased above 85°C, more bonds between heme and globin could have broken, which explained the greater increase in the hematin content in beef and chicken at 100°C.

The gel filtration column separated protein according to their molecule size with a shorter retention time for larger molecules. Ferritin was the earliest iron-containing compound to elute from the column because of its high molecular weight (450,000). Decker and Welch (1990) reported that temperature had a marked effect on the amount of iron released from ferritin by the reducing agents, ascorbate and cysteine. The ascorbate-released iron increased 3.3-fold and cysteine-released iron increased 5-fold as the temperature was increased from 2 to 37°C. For the current experiment, the content of ferritin iron decreased as the temperature

increased. More ferritin iron was decreased in the meat samples from room temperature to 70°C than from room temperature to 55°C. Heat might stimulate the release of iron bound to ferritin by weakening the bond between iron and ferritin, causing a decreased measurement of ferritin iron. The denaturation of ferritin at temperatures higher than 70°C (Seman et al., 1991) resulted in insolubility in water, and so the iron content in ferritin fractions in meat samples heated to 85°C and 100°C could not be determined by the chromatographic technique. The denaturation product of ferritin was probably hemosiderin (Linder, 1988), which was a water-insoluble iron storage complex.

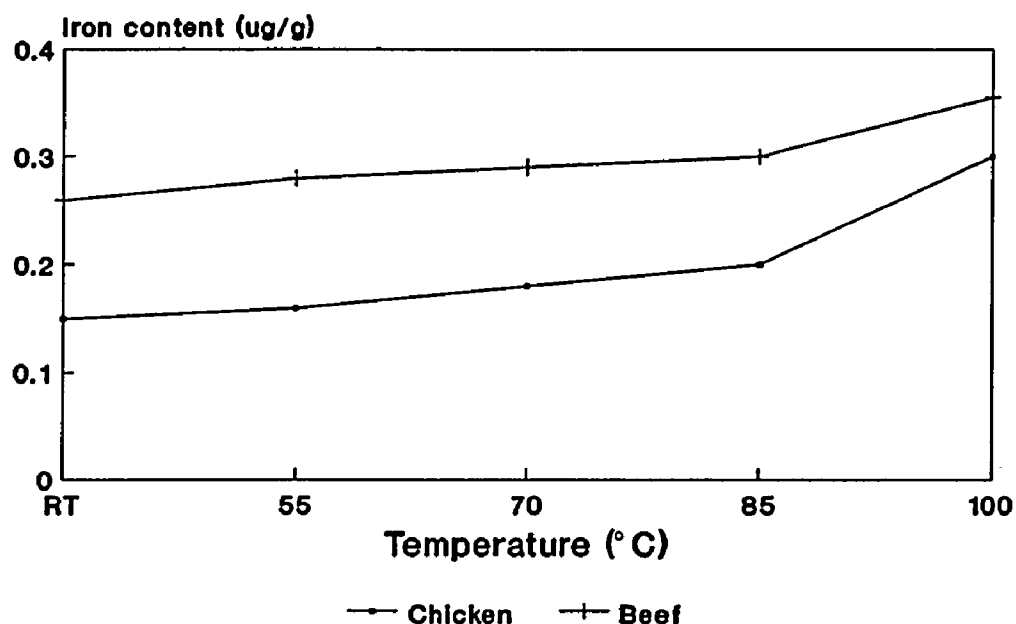


Figure 4.6 Effect of temperature on the contents of hematin iron in beef longissimus and chicken thigh muscles.

In conclusion, heat affected the distribution of iron in different fractions in both beef and chicken muscles. Iron contents in water-soluble fractions were decreased and in water-insoluble fractions were increased as temperature increased to 100°C, with the greatest changes at 70°C. Heat decreased the contents of heme iron and ferritin iron and increased the amount of diffusate iron, i.e. iron in low molecular weight fractions. The increase in diffusate iron by heat might be partially responsible for the rapid development of oxidative rancidity in cooked meat since nonheme iron was reported to be a major catalyst of lipid oxidation in cooked meat (Sato and Hegarty, 1971; Love and Pearson, 1974; Igene et al., 1979). The decrease of heme iron by heat would reduce the bioavailability of meat iron since the absorption of heme iron was reported to be much higher than that of nonheme iron (Monsen et al., 1978; Mahoney, 1986).



## **CHAPTER V**

### **EXPERIMENT THREE: EFFECT OF HEAT AND DIFFERENT FRACTIONS OF IRON ON LIPID OXIDATION**

## INTRODUCTION

Lipid instability is one of the major reasons for quality deterioration in stored meat and meat products. The unsaturated fatty acids, particularly polyunsaturated fatty acids, are oxidized to hydroperoxides by a free radical chain mechanism involving initiation, propagation, and termination stages (Uri, 1961; Lundberg, 1962; Pearson et al., 1983). The hydroperoxides will break down to carbonyls, alcohols and esters, or react with proteins, vitamins, and pigments, all of which are believed to contribute to oxidized flavors and unappealing color in meats (Melton, 1983; Pearson et al., 1983).

Cooked meat developed rancid flavor more rapidly than uncooked meat during refrigerated storage, resulting in "warmed-over flavor" (WOF) (Tims and Watts, 1958). The heating temperature also affected the extent of lipid oxidation in cooked meat. Previous studies indicated that lipid oxidation would increase when the cooking temperature reached 70°C (Keller and Kinsella, 1973; Pearson et al., 1977). However, when the cooking temperature was above 80°C, lipid oxidation tended to decrease (Huang and Greene, 1978; Lillard, 1987).

It has been generally accepted that iron in some form may catalyze the oxidation of lipid in meat (Love, 1987). The heme pigments, myoglobin (Mb) and hemoglobin (Hb), have generally been considered to be the major catalysts of lipid

oxidation in meat, especially in raw red meat (Younathan and Watts, 1959; Tappel, 1962; Greene, 1971; Love, 1983; Rhee, 1988). On the other hand, lipid oxidation in cooked meat has been shown to be accelerated by nonheme iron (Sato and Hegarty, 1971; Love and Pearson, 1974; Igene et al., 1979). However, several researchers found that both heme and nonheme iron have catalytic activity in either raw or cooked meat systems (Wills, 1966; Liu and Watts, 1970). Although research in this area has been conducted for decades, further studies are needed to specify the major catalysts of lipid oxidation in meat.

Iron in meat can exist in a number of different complexes, including low molecular weight compounds, heme compounds, and storage complexes such as ferritin and hemosiderin (Hazell, 1982; Stryer, 1988). Moreover, the distribution of various iron fractions could be altered by heat (Schricker et al., 1982; Chen et al., 1984), which could in turn affect lipid oxidation in cooked meat. Empirical data on the effect of heat and different forms of meat iron on lipid oxidation may help to elucidate the mechanisms involved in WOF.

Since various forms of iron are contained in meat, it is virtually impossible to determine the role of various iron fractions on lipid oxidation in meat in its natural form. Therefore, model systems which should contain either certain form(s) of identified iron or no iron at all are necessary to

determine the role of various iron fractions. The objectives of this study were (1) to determine the role of different forms of meat iron on lipid oxidation in model systems prepared from beef and chicken muscle; and (2) to assess the effect of heating temperatures on lipid oxidation in model systems containing iron fractions.

## **MATERIALS AND METHODS**

### **Source of meat**

Approximately 300 g of bovine longissimus dorsi muscle (pH 5.6) was obtained from each of four beef carcasses at the Louisiana State University Agricultural Center Meat Laboratory. Six refrigerated chickens (weighing approximately 1.8 kg each) were purchased from a local supermarket in Baton Rouge. Bone-in thighs (pH 6.2) were cut from the whole chickens by knife. The muscle samples from each cattle or chicken were vacuum-packaged individually in vinyl bags and stored at  $-18^{\circ}\text{C}$  until being used for analysis (about two and half months for bovine muscle and one week for chicken muscle).

### **Analytical procedures**

The moisture and fat contents in samples were determined by the rapid microwave procedures (AVP80 and Automatic Extraction System, CEM Corporation, Matthews, NC). The total iron content in the samples was determined on wet-ashed samples by atomic absorption spectrophotometry as described in Appendix Method 1.

### **Extraction of muscle tissue and preparation of model system I**

This model system was similar to the one described by Sato and Hegarty (1971). To prepare the model system, approximately 300 g of frozen vacuum-packaged beef from each of the four cattle or 200 g thighs from each of the six

chicken were thawed at room temperature for 2 hours. The visible fat and connective tissues of the beef or chicken samples were trimmed, and the trimmed beef or chicken was cut into small pieces (about 1.25 cm square) with a knife. All beef or chicken samples were then mixed and ground through a 4.7 mm stainless steel plate. A sample of about 100 grams was randomly selected from the ground beef or ground chicken batches individually, vacuum-packaged, labelled as beef or chicken, and stored at  $-18^{\circ}\text{C}$ . This vacuum-packaged ground sample of muscle would be used to assess lipid oxidation for comparison with that in corresponding model systems.

Approximately 1000 grams of beef or chicken samples were randomly taken from the ground beef or chicken, and were homogenized with the same volume of deionized water in a blender (Waring, Hartford, CT) at low speed for 2 minutes. The homogenates were centrifuged at  $3000 \times g$  for 20 minutes. Supernatants were saved, while the residues were re-homogenized with another 1000 ml deionized water before re-centrifugation. A total of four repeated extractions were applied to obtain a nearly colorless insoluble residue. The water-extracted residues were vacuum-packaged with approximately 100-gram in each bag and were frozen immediately for storage at  $-18^{\circ}\text{C}$ . These samples of water-extracted residues were used as model system I for the study of lipid oxidation.

The moisture and fat contents of beef residue were  $66.29 \pm 1.17\%$  and  $2.46 \pm 0.15\%$ , respectively. Chicken residue had moisture and fat contents of  $87.47 \pm 0.03\%$  and  $2.38 \pm 0.08\%$ , respectively. The iron contents in beef and chicken model system I were 8.01 and 9.12  $\mu\text{g/g}$ , respectively, which approximated the water-insoluble iron content in corresponding bovine longissimus muscle and chicken thigh as determined in Chapter IV.

#### Preparation of model system II

Emulsions of beef or chicken intramuscular fat were used as model system II substrates for oxidation studies and were always prepared immediately before use. Beef or chicken intramuscular fat was extracted by organic solvents from the corresponding water-extracted meat residues by the method of Folch et al. (1957) (Appendix Method 2). The reason for extracting fat from the water-extracted meat residue, instead of raw meat, was to prevent heme iron from being present in the fat. Hemoproteins which contain heme iron were removed during water extraction. The stable emulsions were obtained by mixing 9 volumes of 0.025 M orthophosphate buffer (pH 6.0) with 1 volume of a solution of fat in ethanol (the fat concentration in ethanol was 40.5% for beef and 32.6% for chicken) in a blender (Waring, Hartford, CT) at low speed for 30 seconds (Wills, 1965). The percentages of fat in bovine and chicken model system II were 4.05 and 3.26%, respectively, which corresponded to the fat contents in raw

bovine longissimus muscle or chicken thigh. The iron content in beef and chicken model system II were 0.08 and 0.06  $\mu\text{g/ml}$ , respectively, which could have been from either the intramuscular fat or phosphate buffer used to prepare this model system.

#### **Fractionation of iron-containing water soluble components of muscle**

Supernatants obtained in the preparation of model system I were combined, freeze-dried (Consol 24 freeze drier, Virtis, Gardiner, N.Y.), and retained for fractionation. Because it would take very long time to freeze-dry all supernatants from the four extractions of a meat sample, only supernatants from the first two extractions were combined and freeze-dried. The freeze-dried water-soluble extracts were fractionated into ferritin, Hb, and Mb fractions on a gel filtration column (Ultrogel ACA 34, Spectrum, Los Angeles, CA) by procedures described by Hazell (1982). Three grams of the freeze-dried extract were dissolved in 15 ml of deionized water. Ten ml of each solution were loaded onto the column (height 100 cm, width 2.5 cm, Bio-Rad, Richmond, CA). In order to determine the retention times of ferritin, Hb, and Mb fractions from the column, an identification experiment was conducted. Bovine Mb (purified from beef by the method of Yamazaki et al., 1964), bovine Hb (Sigma Chemical Co., St. Louis, MO) and ferritin from horse spleen (Sigma Chemical Co., St. Louis, MO) were used as standards to identify the



ferritin, Hb, and Mb peaks of the samples. A 10-ml mixture of ferritin, Hb and Mb standard solution or sample extract was loaded on the column and eluted with 0.2 M potassium orthophosphate buffer (pH 6.8) at a flow rate of 0.42 ml/minute (Hazell, 1982). At 12 hours after loading of the sample, fractions were collected at 2-minute intervals. The absorption of collected fractions were measured at 540 nm by a spectrophotometer (Model U-2000 UV/Vis Spectrophotometer, Danbury, CT) to determine the existence of ferritin, Hb, or Mb in each fraction. The chromatograms of the standard mixture and the aqueous extracts of beef and chicken muscles on the gel filtration column are shown in Appendix Figure 8. Ferritin standard was the earliest iron fraction being eluted from the column, followed by hemoglobin, and then myoglobin fractions (Appendix Figure 8). A peak from the sample which had a similar retention time to a peak of the standard was considered to be from a compound in the sample similar to the standard. Since each peak of the samples was identified by comparing its retention time with the peak of a corresponding standard, the purify of each peak from the samples was not further affirmed. Therefore, ferritin, Hb, and Mb of the samples separated from the gel filtration column were called corresponding fractions instead of individual compound.

Because the retention times of ferritin, Hb and Mb were identified, it was decided that a 10-minute interval was appropriate for the collection of fractions from samples for

the ferritin, Hb and Mb separation. Fractions containing the same compound (two fractions collected from 10 to 20 minutes, and 20 to 30 minutes for ferritin; two fractions collected from 30 to 40, and 40 to 50 minutes for Hb; and three fractions collected from 50 to 60, 60 to 70, and 70 to 80 minutes for Mb) (Appendix Figure 8) were pooled, freeze-dried, and stored in a desiccator at 4°C.

Diffusate and non-diffusate iron fractions were prepared by dissolving 3 g of freeze-dried water soluble extract in 15 ml deionized water. Ten ml of each solution was dialyzed against 60 ml of deionized water at 4°C for 48 hours by using 4.5 cm standard dialysis tubing (molecular weight cutoff 12,000 daltons) (Fisher Scientific, Pittsburgh, PA). The diffusate (dialyzable fraction) and non-diffusate fractions were then freeze-dried and stored in a desiccator at 4°C. Iron content in each freeze-dried fraction was determined by atomic absorption spectrophotometry, as described in Appendix Method 1.

#### **Treatment of iron for model systems**

The catalytic ability of nine forms of iron were tested in the experiment. These irons were ferrous chloride (Sigma Chemical Co., St. Louis, MO), ferric chloride (Sigma Chemical Co., St. Louis, MO), hematin (Sigma Chemical Co., St. Louis, MO), water-extractable fraction, ferritin fraction, hemoglobin fraction, myoglobin fraction, diffusate fractions, and non-diffusate fractions. All were common forms of iron

which existed in meat except ferrous chloride and ferric chloride. The inclusion of ferrous chloride and ferric chloride was intended to determine which oxidative state of iron, ferrous or ferric, would have a higher catalytic ability on lipid oxidation.

Each of the 9 forms of iron was added independently into each of the 2 model systems for either beef or chicken. The concentration of each iron form added to beef or chicken model systems is listed in Table 5.1, which corresponded to the mean values of iron content for each component in the raw beef or chicken samples determined in Chapter IV. Because the exact concentrations of free  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  in beef and chicken muscle were unknown, the amount of  $\text{Fe}^{2+}$  or  $\text{Fe}^{3+}$  added in the model systems was based on the content of diffusate iron in beef or chicken muscle. Diffusate iron represents the iron in the low molecular weight fraction.

The desired amount of each iron fraction was accurately weighed (Mettler AT 261 Balance, Greifensee, Switzerland) and mixed with 100 g of the corresponding model system in a blender (Waring, Hartford, CT) for 30 seconds to evenly distribute the iron in the model systems. The iron-mixed model system was divided into five portions of approximately 20 g each into 50-ml Erlenmeyer flasks. The flasks were stoppered with rubber stoppers before heating.

### Method of heating

Four levels of heating temperature, 55°C, 70°C, 85°C, and 100°C, were selected in the experiment. The rationale for selecting the 4 heating temperatures was discussed in Chapter IV (p. 58). The flasks with samples were placed inside 250-ml beakers in a steam water bath at 101.6°C (Precision Scientific Co., Chicago, IL). The temperature of the water bath was higher than 100°C due to the existence of steam in the container to produce pressure. Four flasks with samples were placed in the steam water bath side by side. A thermocouple was placed in the center of the sample in each flask through a perforated hole of the stopper to detect temperature changes. The thermocouples were linked to the

**Table 5.1 Concentration of each iron fraction added to beef or chicken model system I and II.**

Iron Type	Beef MS II or I	Chicken MS II or I
H <sub>2</sub> O extractable Fe	17.0 ppm	5.5 ppm
Diffusate Fe	1.75 ppm	1.0 ppm
Non-diffusate Fe	15.3 ppm	4.5 ppm
Hematin	2.7 ppm	1.7 ppm
Hemoglobin	0.8 mg/g	0.2 mg/g
Myoglobin	3.5 mg/g	0.12 mg/g
Ferritin	0.4 ppm	0.7 ppm
FeCl <sub>2</sub>	1.75 ppm	1.0 ppm
FeCl <sub>3</sub>	1.75 ppm	1.0 ppm

Digital Data Acquisition System (MackMac 1240-Touch Display, GreenSpring Computers, Menlo Park, CA) which monitored the temperature change by using the Thermo VI program. One flask was removed randomly from the water bath when the internal temperatures reached 55°C, 70°C, 85°C, or 100°C. The removed flasks were cooled to room temperature (27°C) in ice water. The samples were stored at 4°C for 48 hr before assessment of lipid oxidation. Any changes in color and odor of the samples were not obvious after 48 hr refrigerated storage.

The heating curves of model system I and II in the water bath are given in Appendix Figure 9. Model system I was the water-extracted beef or chicken residue which was in semi-solid state. Model system II was the beef or chicken fat emulsion which was in a liquid state. Heat transferred faster in the liquid model system II than in the semisolid model system I. The time periods required to reach the internal temperatures of 55°C, 70°C, 85°C, and 100°C in model system I were 2.5, 4.5, 6.7, and 20.8 minutes, respectively. The time periods required to reach the internal temperatures of 55°C, 70°C, 85°C, and 100°C in model system II were 1.8, 3.5, 5.2, and 12.0 minutes, respectively.

Two experiment replications were performed for each combination of iron and temperature treatment in each model system for either beef or chicken. Two samples were selected from each of the treatment combinations to determine the

extent of lipid oxidation. Corresponding model systems with no iron added were used as control groups.

Ground bovine longissimus muscle or chicken thigh used to prepare the water-extracted residues was also assessed for lipid oxidation after being heated to the four temperatures and stored at 4°C for 48 hr. The extent of lipid oxidation in beef and chicken meat was compared with that in corresponding iron added model systems.

#### **Determination of lipid oxidation**

The extent of development of oxidative rancidity in samples was evaluated by the 2-thiobarbituric acid (TBA) test (Appendix Method 3) (Tarladgis et al., 1960) and peroxide value (PV) test (Appendix Method 4) (Koniecko, 1985). The TBA values were expressed as milligrams of malondialdehyde per kilogram of the sample. The peroxide values were expressed as milliequivalents of peroxide per kilogram of the sample.

#### **Determination of fatty acids in model systems**

Fats were extracted from the model systems by applying the method of Folch et al. (1957) (Appendix Method 2). The fatty acid methyl esters (FAME) of the total lipids were prepared by using the method described by Metcalfe et al. (1966) (Appendix Method 5). The percentage of each methyl ester in model system was determined by gas chromatography at the USDA-ARS Southern Regional Research Center Food Quality Laboratory in New Orleans.

### **Statistical analysis**

The experiment of this study was a split-plot design with two replications. The type of iron was treated as the whole plot; and the temperature was treated as the sub-plot. Analyses of variance (SAS, 1985b) with main effects of temperature and type of iron added were performed for dependent variables of TBA or PV. Least-squares means were compared by using t-tests when analyses of variance indicated treatment effects ( $P < 0.05$ ). Pearson correlation was performed to determine relationship between TBA and PV values.

## RESULTS AND DISCUSSION

The objective of the experiment was to determine the role of various forms of iron in muscle on lipid oxidation. Since muscle contains all these forms of iron, model systems were utilized to study catalysis of lipid oxidation in muscle foods. Model system I was the water-extracted residue of beef or chicken muscle, and model system II was the emulsion of beef or chicken intramuscular fat.

### Fatty acid comparison of model systems

The fatty acid composition of model systems I and II of beef and chicken are shown in Table 5.2. Generally, model system I had a higher proportion of unsaturated fatty acids than model system II for both beef and chicken. Chicken model systems contained more unsaturated fatty acids and polyunsaturated fatty acids than beef model systems. The predominate saturated fatty acids in both beef and chicken model systems were palmitate and stearate. The proportion of palmitate was similar between beef and chicken model systems, while the percentage of stearate was much higher in beef than in chicken model systems. The predominate unsaturated fatty acids in beef model systems were oleate and palmitoleate, while oleate, linoleate, and palmitoleate were the predominate unsaturated fatty acids in chicken model systems. Model systems I had a higher percentage of oleate than model systems II. Linoleate was almost the only polyunsaturated fatty acid in both beef and chicken model systems. The



percentage of linoleate was much higher in chicken than in beef model systems.

**Table 5.2** Least-squares means<sup>1</sup> for percentage composition of fatty acids in beef and chicken model systems (MS).

Fatty acid component		Beef		Chicken	
Name	Number of Carbon	MSI	MSII	MSI	MSII
Myristate	14:0	1.83 <sup>b</sup>	3.81 <sup>a</sup>	0.63 <sup>c</sup>	1.27 <sup>bc</sup>
-	15:0	0.41 <sup>a</sup>	2.05 <sup>a</sup>	0.31 <sup>a</sup>	1.44 <sup>a</sup>
Palmitate	16:0	22.10 <sup>a</sup>	23.86 <sup>a</sup>	22.67 <sup>a</sup>	23.89 <sup>a</sup>
Palmitoleate	16:1	3.31 <sup>a</sup>	10.42 <sup>a</sup>	7.77 <sup>a</sup>	8.33 <sup>a</sup>
Margarate	17:0	1.93 <sup>b</sup>	5.27 <sup>a</sup>	0.24 <sup>b</sup>	0.39 <sup>b</sup>
Stearate	18:0	21.23 <sup>a</sup>	18.56 <sup>b</sup>	5.50 <sup>c</sup>	5.54 <sup>c</sup>
Oleate	18:1	46.83 <sup>a</sup>	35.44 <sup>c</sup>	41.32 <sup>ab</sup>	38.79 <sup>bc</sup>
Linoleate	18:2	1.60 <sup>c</sup>	1.43 <sup>c</sup>	20.01 <sup>a</sup>	19.04 <sup>b</sup>
Linolenate	18:3	0.21	-	-	-
Arachidate	20:0	0.15 <sup>a</sup>	-	0.12 <sup>a</sup>	-
-	20:1	0.49 <sup>b</sup>	4.12 <sup>a</sup>	0.61 <sup>b</sup>	0.46 <sup>b</sup>
-	21:0	0.56 <sup>b</sup>	1.39 <sup>ab</sup>	0.89 <sup>b</sup>	1.66 <sup>a</sup>
-	23:0	0.07 <sup>b</sup>	-	0.25 <sup>a</sup>	0.23 <sup>a</sup>
Total SFA <sup>2</sup>		48.18 <sup>b</sup>	52.19 <sup>a</sup>	30.62 <sup>d</sup>	33.47 <sup>c</sup>
Total UFA <sup>2</sup>		52.27 <sup>c</sup>	47.81 <sup>d</sup>	69.50 <sup>a</sup>	66.53 <sup>b</sup>
Total PUFA <sup>2</sup>		1.64 <sup>c</sup>	1.42 <sup>c</sup>	20.01 <sup>a</sup>	19.04 <sup>b</sup>

<sup>1</sup>Each value was the least-squares means of five repeated measurements from the same sample.

<sup>2</sup>SFA = saturated fatty acids; UFA = unsaturated fatty acids; PUFA = polyunsaturated fatty acids.

<sup>abcd</sup>Means not bearing same superscripts on the same row are different ( $p > 0.05$ ).

#### Effect of heat and various forms of iron on lipid oxidation in beef or chicken model system I

Model system I used in the experiment was the water-extracted beef or chicken residue. In preparing the model system, water was used to remove most pigments, other forms of water-extractable iron, and minerals from the meat sample

(Sato and Hegarty, 1971; Chen et al., 1984). The prepared water-extracted meat residue contained water-insoluble iron, water-insoluble proteins, and lipids.

Results from analyses of variance of TBA or PV in beef model system I indicated that lipid oxidation in the model system was affected ( $p < 0.05$ ) by both of the treatments of heat and the type of iron added (Appendix Tables 5 and 6). Interactions ( $p < 0.05$ ) also existed between the heating temperature and the type of iron added in the beef model system I by the measurements of both TBA and PV, which meant that heat had different effect on lipid oxidation in model system I catalyzed by various forms of iron.

The extent of lipid oxidation in model systems was assessed by measuring PV and TBA values. The peroxide value test (PV) measures the primary oxidative products, or the peroxides (Koniecko, 1985). The TBA test determines the content of the secondary oxidative product, or malondialdehyde, which is one of the breakdown products of the peroxides (Tarladgis et al., 1960).

Results of the TBA and PV values in the beef model system I catalyzed by heat and different forms of iron are summarized in Tables 5.3 and 5.4, respectively. The results showed a similar rank order in the catalytic ability on lipid oxidation of iron fractions in the measurements of PV and TBA values. The Pearson correlation coefficient between TBA and PV in beef model system I was 0.84. The high correlation

between TBA and PV suggested that the catalytic abilities of iron fractions on the formation of primary and secondary oxidative products were similar.

**Table 5.3** Effect of heat and various iron fractions on lipid oxidation expressed as TBA values<sup>1,2</sup> in water-extracted beef residue (model system I) stored at 4°C for 48h.

Iron Treatment	Temperature				
	Raw	55°C	70°C	85°C	100°C
Control	0.58 <sup>a</sup>	0.92 <sup>b</sup>	1.28 <sup>c</sup>	1.56 <sup>d</sup>	1.50 <sup>d</sup>
H <sub>2</sub> O Extractable Fe	2.66 <sup>b</sup>	2.93 <sup>j</sup>	3.59 <sup>l</sup>	4.05 <sup>n</sup>	3.90 <sup>p</sup>
Diffusate Fe	2.14 <sup>g</sup>	2.57 <sup>h</sup>	2.98 <sup>j</sup>	3.47 <sup>l</sup>	3.36 <sup>o</sup>
Non-diffusate Fe	1.98 <sup>f</sup>	2.14 <sup>g</sup>	2.62 <sup>i</sup>	3.19 <sup>k</sup>	3.10 <sup>n</sup>
Hematin	1.13 <sup>d</sup>	1.57 <sup>e</sup>	1.93 <sup>f</sup>	2.80 <sup>h</sup>	2.88 <sup>i</sup>
Hemoglobin	1.64 <sup>e</sup>	1.93 <sup>f</sup>	2.52 <sup>h</sup>	3.13 <sup>jk</sup>	3.04 <sup>lm</sup>
Myoglobin	1.60 <sup>e</sup>	1.90 <sup>f</sup>	2.58 <sup>i</sup>	3.08 <sup>j</sup>	3.01 <sup>l</sup>
Ferritin	0.83 <sup>c</sup>	1.60 <sup>e</sup>	2.12 <sup>g</sup>	2.78 <sup>h</sup>	3.09 <sup>n</sup>
FeCl <sub>2</sub>	4.32 <sup>b</sup>	4.87 <sup>k</sup>	6.01 <sup>m</sup>	6.59 <sup>o</sup>	6.99 <sup>q</sup>
FeCl <sub>3</sub>	0.75 <sup>b</sup>	2.64 <sup>i</sup>	3.50 <sup>k</sup>	3.85 <sup>m</sup>	3.86 <sup>mp</sup>
Beef <sup>3</sup>	2.83	3.15	3.86	4.28	4.01

<sup>1</sup>Each TBA value (mg malondialdehyde/kg sample) is the least-squares means of four measurements.

<sup>2</sup>Standard error of mean = 0.02.

<sup>3</sup>Ground longissimus muscle with standard error of mean=0.01.

Means in the same row or column not bearing a common superscript letter are different (p<0.05).

The results on lipid oxidation also indicated that the TBA and PV values of beef residue with water-extractable iron were similar to those obtained from the ground longissimus

muscle. These findings suggested that water-extractable fraction was the major catalysts of lipid oxidation, while water insoluble iron had very limited catalytic ability on

**Table 5.4** Effect of heat and various iron fractions on lipid oxidation expressed as PV<sup>1,2</sup> in water-extracted beef residue (model system I) stored at 4°C for 48h.

Iron Treatment	Temperature				
	Raw	55°C	70°C	85°C	100°C
Control	2.64 <sup>a</sup>	4.69 <sup>a</sup>	9.46 <sup>b</sup>	11.01 <sup>b</sup>	10.75 <sup>b</sup>
H <sub>2</sub> O Soluble Fe	9.64 <sup>c</sup>	12.79 <sup>ij</sup>	16.88 <sup>l</sup>	19.72 <sup>or</sup>	19.22 <sup>rvw</sup>
Diffusate Fe	8.72 <sup>c</sup>	11.42 <sup>gi</sup>	15.18 <sup>kl</sup>	18.25 <sup>mop</sup>	17.80 <sup>puvw</sup>
Non-diffusate Fe	8.68 <sup>cc</sup>	10.79 <sup>fgi</sup>	15.01 <sup>kl</sup>	17.61 <sup>mo</sup>	16.96 <sup>otuv</sup>
Hematin	5.36 <sup>b</sup>	10.31 <sup>fgh</sup>	12.01 <sup>hi</sup>	14.92 <sup>l</sup>	15.06 <sup>h</sup>
Hemoglobin	7.84 <sup>cd</sup>	9.96 <sup>dfg</sup>	14.17 <sup>ijk</sup>	17.08 <sup>lmn</sup>	16.70 <sup>ntu</sup>
Myoglobin	7.79 <sup>c</sup>	10.15 <sup>cfg</sup>	14.21 <sup>ijk</sup>	17.00 <sup>lm</sup>	16.69 <sup>mtu</sup>
Ferritin	4.25 <sup>ab</sup>	8.78 <sup>f</sup>	13.09 <sup>ij</sup>	15.01 <sup>jl</sup>	16.35 <sup>tu</sup>
FeCl <sub>2</sub>	11.38 <sup>f</sup>	14.56 <sup>j</sup>	20.75 <sup>m</sup>	26.13 <sup>s</sup>	26.92 <sup>s</sup>
FeCl <sub>3</sub>	4.63 <sup>ab</sup>	10.29 <sup>fg</sup>	16.05 <sup>kl</sup>	19.64 <sup>oq</sup>	19.73 <sup>qw</sup>
Beef <sup>3</sup>	9.99	13.63	18.60	20.68	19.92

<sup>1</sup>Each PV (milliequivalents of peroxide per 1000 gm. of sample) is the least-squares means of four measurements.

<sup>2</sup>Standard error of mean = 0.83.

<sup>3</sup>Ground longissimus muscle with standard error of mean=0.51.

Means in the same row or column not bearing a common superscript letter are (p<0.05).

the oxidation of lipid in beef. The finding from the current experiment was in agreement with Apte and Morrissey (1987b), who reported that the water-extracted muscle system

containing added total soluble iron gave TBA values similar to those obtained for the intact muscle systems.

The extent of lipid oxidation in the raw control sample (no heat and no iron added beef model system I) was lower ( $p < 0.05$ ) than those in most of the iron and heat treatment samples of the beef model system I samples (Tables 5.3 and 5.4). So, lipid oxidation was promoted by increasing the heating temperature and/or adding iron fractions in this model system. PV and TBA values in the model system I of each iron added sample showed increases as the heating temperature increased to 85°C (Tables 5.3 and 5.4). Moreover, the PV and TBA values in the beef residue catalyzed by  $\text{FeCl}_2$ ,  $\text{FeCl}_3$ , ferritin fraction, or hematin continued to increase as the heating temperature increased from 85°C to 100°C. However, lipid oxidation for the beef residues containing the other iron fractions was decreased ( $p < 0.05$ ) when the heating temperature increased from 85°C to 100°C.

Heat might have induced changes in microsomes, mitochondria, and membranes, causing them to be more susceptible to oxidation (Cross et al., 1987). Heat might also disrupt muscle membranes and resulted in exposure of more labile lipid components to oxygen and other reaction catalysts (Sato and Hegarty, 1971). On the other hand, the Maillard reaction, which is the nonenzymatic, browning reaction between amino acids or proteins and carbohydrates, can take place in meats at high temperature (Bailey et al.,

1987). Several researchers indicated that the Maillard reaction products (MRP), such as melanoidins and their precursors, reductones, had strong antioxidant properties in cooked meats (Hamm, 1966; Sato et al., 1973; Huang and Greene, 1978; Bailey et al., 1987). According to Hamm (1966), the Maillard reactions in meats began at about 90°C and increased with increased temperature and heating time. Therefore, lipid oxidation at 100°C in meat residues were determined by two opposite factors: the catalytic factor by iron and heat, and the prevention factor by MRP. The catalytic ability of ferritin,  $\text{Fe}^{2+}$ , or  $\text{Fe}^{3+}$  might be great enough to exceed the antioxidant effect of MRP which resulted in slight increase of TBA and PV values in beef residue even at 100°C. Reductones and similar compounds are excellent hydrogen donors. The radical intermediates of reductones and similar compounds are relatively stable due to resonance delocalization and to lack of positions suitable for attack by molecular oxygen (Bailey et al., 1987). The antioxidative effect of the reductones and similar compounds was probably due to the transfer of hydrogen atoms to hydroperoxy radicals to prevent further formation of free radicals from unsaturated fatty acids (Eichner, 1979; Bailey et al., 1987).

The results (Tables 5.3 and 5.4) also indicated that catalytic ability of  $\text{Fe}^{3+}$  and ferritin in raw beef residues were very low. But heat could promoted lipid oxidation in the  $\text{Fe}^{3+}$  or ferritin added meat residues ( $P < 0.05$ ). Apte and

Morrissey (1987a) reported that intact ferritin was not a catalyst of lipid oxidation in meat. Heat could cause the release of  $\text{Fe}^{3+}$  from ferritin which then catalyzed lipid oxidation.

The lipid oxidation in meat residues catalyzed by Hb fraction, Mb fraction, or hematin also increased ( $p < 0.05$ ) as the heating temperature increased (Table 5.3 and 5.4). The biggest increase of TBA and PV took place in the beef residue when the heating temperatures ranged from  $55^{\circ}\text{C}$  to  $85^{\circ}\text{C}$ . It was found in previous studies that the greatest hemoprotein denaturation and heme iron reduction occurred within this temperature range (Chapter IV). These results suggested that the increases in the oxidation of lipid by hemoprotein as a result of heat treatment might be caused by both the conformation changes of the hemoproteins, which resulted in greater exposure of catalytic heme group to unsaturated fatty acids (Greene and Price, 1975; Igene et al., 1979), and the release of nonheme iron from heme (Chen et al., 1984).

$\text{FeCl}_2$  had the highest TBA and PV values among all forms of iron (Tables 5.3 and 5.4), which suggested that  $\text{FeCl}_2$  had the strongest catalytic ability in lipid oxidation in both raw and cooked beef model system I. However, TBA or PV values in the beef residue catalyzed by diffusate iron were much lower than these catalyzed by  $\text{Fe}^{2+}$ . This might be caused by the presence of oxidation inhibitors (e.g. glutathione, cysteine, and histidine) in the diffusate

fraction (Sato and Hegarty, 1971; Decker and Schanus, 1986) and/or the presence of a greater proportion of  $\text{Fe}^{3+}$  in the diffusate fraction. Despite the presence of oxidation inhibitors, lipid oxidation in the beef residues catalyzed by diffusate iron was as high as or higher than that catalyzed by non-diffusate iron at the same heating temperature (Tables 5.3 and 5.4), suggesting that iron in the low molecular weight fraction (diffusate iron) might have a greater catalytic effect than heme iron. However, heme iron also exerted major catalytic ability in the meat residues (Tables 5.3 and 5.4).

The catalytic abilities of Mb and Hb fractions were similar in the beef model system I. To compare with Mb and Hb fractions, hematin had a lower catalytic ability in the model systems, which might due to its lower concentration in the model system. The catalytic activity of heme iron found in the experiment was different from the findings by Sato and Hegarty (1971), who observed no acceleration of lipid oxidation when hemoglobin and myoglobin were added to water-extracted muscle residues at levels of 10-25 and 5 mg/g muscle, respectively. The high concentration of heme pigments in their muscle residues might function as an oxidation inhibitor rather than catalyst (Fox and Benedict, 1987).

In the current experiment, non-diffusate iron included ferritin, Hb and Mb fractions. The TBA or PV values in model



system I catalyzed by non-diffusate iron were much less than the sum of the TBA or PV catalyzed by ferritin, Hb, and Mb fractions (Tables 5.3 and 5.4). The sum of TBA or PV in beef residue catalyzed by diffusate and non-diffusate iron was also unequal to that catalyzed by water extractable iron, suggesting that the catalytic effect of iron was not additive. Therefore, determination of the relative contribution of different fractions of iron to lipid oxidation was impossible because of the non-additive catalytic ability of iron and the possible presence of oxidation inhibitors in the diffusate fraction.

Results from analyses of variance of TBA or PV in chicken model system I indicated that lipid oxidation in the model system was affected ( $p < 0.05$ ) by both the heating temperature and the type of iron added (Appendix Tables 5 and 6). Interactions ( $p > 0.05$ ) existed between the heating temperature and the type of iron added in the chicken model system I in both of the TBA and PV measurements, which meant that heat had different effect on lipid oxidation in model system I catalyzed by various forms of iron.

The results of TBA and PV values in the chicken model system I catalyzed by heat and different forms of iron are summarized in Tables 5.5 and 5.6, respectively. A Pearson correlation coefficient of 0.85 was calculated between the TBA and PV values in chicken model system I, indicating the two measurements highly correspond to each other. The extent

**Table 5.5** Effect of heat and various iron fractions on lipid oxidation expressed as TBA values<sup>1,2</sup> in water-extracted chicken thigh residue (model system I) stored at 4°C for 48h.

Iron Treatment	Temperature				
	Raw	55°C	70°C	85°C	100°C
Control	0.80 <sup>a</sup>	1.18 <sup>b</sup>	1.51 <sup>c</sup>	1.77 <sup>d</sup>	1.65 <sup>d</sup>
H <sub>2</sub> O Soluble Fe	2.94 <sup>e</sup>	4.49 <sup>b</sup>	6.69 <sup>j</sup>	8.78 <sup>m</sup>	8.49 <sup>n</sup>
Diffusate Fe	2.69 <sup>f</sup>	4.22 <sup>e</sup>	6.26 <sup>i</sup>	8.42 <sup>l</sup>	8.08 <sup>m</sup>
Non-diffusate Fe	2.33 <sup>e</sup>	4.05 <sup>g</sup>	6.07 <sup>i</sup>	8.15 <sup>k</sup>	7.87 <sup>l</sup>
Hematin	1.90 <sup>d</sup>	2.26 <sup>e</sup>	4.11 <sup>f</sup>	4.78 <sup>hi</sup>	4.71 <sup>i</sup>
Hemoglobin	2.03 <sup>d</sup>	3.83 <sup>f</sup>	5.82 <sup>h</sup>	7.75 <sup>j</sup>	7.43 <sup>k</sup>
Myoglobin	2.02 <sup>d</sup>	3.84 <sup>f</sup>	5.68 <sup>h</sup>	7.63 <sup>j</sup>	7.44 <sup>jk</sup>
Ferritin	1.23 <sup>b</sup>	2.45 <sup>e</sup>	4.53 <sup>g</sup>	4.87 <sup>h</sup>	4.83 <sup>h</sup>
FeCl <sub>2</sub>	4.88 <sup>b</sup>	5.58 <sup>i</sup>	7.09 <sup>k</sup>	9.14 <sup>o</sup>	9.14 <sup>o</sup>
FeCl <sub>3</sub>	1.64 <sup>c</sup>	2.31 <sup>e</sup>	4.06 <sup>f</sup>	4.14 <sup>g</sup>	3.99 <sup>g</sup>
Chicken <sup>3</sup>	3.24	4.78	6.90	9.02	8.57

<sup>1</sup>Each TBA value (mg malondialdehyde/kg sample) is the least-squares means of four measurements.

<sup>2</sup>Standard error of mean = 0.08.

<sup>3</sup>Ground chicken thigh with standard error of mean = 0.07.

<sup>abcde fghijklmno</sup> Means in the same row or column not bearing a common superscript letter are different (p<0.05).

of lipid oxidation in the raw control sample (no heat and no iron added chicken model system I) was lower (p<0.05) than that in most of the iron and/or heat treated chicken model system I. Therefore, the treatment by either heat or iron, or the combination of both could promoted lipid oxidation in the model system. The TBA and PV in the model system I of

**Table 5.6** Effect of heat and various iron fractions on lipid oxidation expressed as PV<sup>1,2</sup> in water-extracted chicken thigh residue (model system I) stored at 4°C for 48h.

Iron Treatment	Temperature				
	Raw	55°C	70°C	85°C	100°C
Control	4.83 <sup>a</sup>	7.87 <sup>b</sup>	11.26 <sup>c</sup>	13.22 <sup>d</sup>	13.15 <sup>d</sup>
H <sub>2</sub> O Soluble Fe	14.99 <sup>f</sup>	18.82 <sup>gh</sup>	25.69 <sup>kl</sup>	30.84 <sup>mn</sup>	29.46 <sup>n</sup>
Diffusate Fe	12.73 <sup>de</sup>	17.59 <sup>fg</sup>	24.11 <sup>jk</sup>	29.63 <sup>lm</sup>	28.60 <sup>m</sup>
Non-diffusate Fe	11.97 <sup>d</sup>	16.82 <sup>f</sup>	23.45 <sup>ij</sup>	28.46 <sup>jl</sup>	27.71 <sup>l</sup>
Hematin	8.86 <sup>c</sup>	15.09 <sup>e</sup>	17.31 <sup>ef</sup>	20.96 <sup>i</sup>	20.70 <sup>h</sup>
Hemoglobin	11.50 <sup>d</sup>	16.01 <sup>ef</sup>	22.37 <sup>hi</sup>	27.68 <sup>j</sup>	27.06 <sup>j</sup>
Myoglobin	11.27 <sup>d</sup>	15.95 <sup>ef</sup>	21.63 <sup>h</sup>	27.85 <sup>jk</sup>	26.64 <sup>k</sup>
Ferritin	6.48 <sup>ab</sup>	12.75 <sup>d</sup>	19.57 <sup>g</sup>	21.81 <sup>fi</sup>	21.39 <sup>fh</sup>
FeCl <sub>2</sub>	14.13 <sup>ef</sup>	21.45 <sup>h</sup>	26.77 <sup>i</sup>	32.14 <sup>no</sup>	32.55 <sup>o</sup>
FeCl <sub>3</sub>	7.56 <sup>bc</sup>	12.98 <sup>d</sup>	15.95 <sup>e</sup>	17.28 <sup>ch</sup>	16.61 <sup>c</sup>
Chicken <sup>3</sup>	15.91	19.55	26.66	31.22	29.69

<sup>1</sup>Each PV (milliequivalents of peroxide per 1000 gm. of sample) is the least-squares means of four measurements.

<sup>2</sup>Standard error of mean = 0.61.

<sup>3</sup>Ground chicken thigh with standard error of mean = 0.68.

<sup>abcde fghijklmno p</sup>Means in the same row or column not bearing a common superscript letter are different (p<0.05).

each iron added sample were increased (p<0.05) as the heating temperature increased up to 85°C but decreased as the temperature was from 85°C and 100°C (Tables 5.5 and 5.6). The Maillard reaction products (MRP), which might form in the chicken residue at temperature higher than 90°C, might be

responsible for the decreased lipid oxidation in the chicken model system I being heated to 100°C.

The catalytic ability on lipid oxidation of the various iron fractions in raw chicken model system I could be rank ordered as the following:  $\text{FeCl}_2$  > water-extractable iron > diffusate iron > non-diffusate iron > Hb and Mb fractions > hematin >  $\text{FeCl}_3$  > ferritin (Tables 5.5 and 5.6). This order of the catalytic ability of the various iron fractions was the same in cooked chicken model system I with the exception of the ferritin fraction. The catalytic ability of ferritin fraction increased close to hematin's in cooked chicken residue.

To compare with the results from beef model system I, same orders were followed for the catalytic ability of iron fractions in the respective raw or cooked chicken model system I. However, the extent of lipid oxidation in chicken model system I appeared to be much higher than that in beef model system I in each heat and iron treatment combination. No attempts of statistical analysis were made to compare TBA or PV values between the beef and chicken model systems because of the violation in the assumptions for such a analysis (different error terms were calculated between the two model systems). The proportion and degree of unsaturated fatty acids in meat could determine the approximate susceptibility of the product to oxidative deterioration. Generally, the higher the proportion and degree of

unsaturation of the fatty acids, the more susceptible the product is to be oxidized (Dawson and Gartner, 1983). The percentages of unsaturated fatty acids in chicken and beef model system I were 69.5 and 47.81 with polyunsaturated fatty acids of 20.02 and 1.64, respectively (Table 5.2). Linoleic acid was almost the only polyunsaturated fatty acid in both chicken and beef model system I. Autoxidized linoleate was reported to produce much more malondialdehyde than from autoxidized oleate (Kenaston et al., 1955; Kwon and Olcott, 1966). Therefore, the higher content of linoleic acid might be responsible for the higher degree of oxidation in the chicken model system I compared to the beef model system I.

**Effect of heat and various forms of iron on lipid oxidation in beef or chicken model system II**

Model system II was developed to study the catalytic ability of iron fractions on the oxidation of lipid in the absence of water-insoluble proteins and muscle fiber. Beef or chicken intramuscular fat was extracted by organic solvents from the corresponding water-extracted beef or chicken residue. Animal intramuscular fat cells are in close proximity to the lean tissue and thus could be expected to be more important in the development of WOF than other adipose tissue (Cross et al., 1987).

Results from the analyses of variance (Appendix Tables 5 and 6) for TBA or PV indicated that both type of iron and heat affected ( $p < 0.05$ ) lipid oxidation in beef model system

II. Interactions ( $p < 0.05$ ) existed between the heating temperature and the type of iron catalyst for the TBA values (Appendix Table 5). Such interaction suggested that the catalytic ability of various forms of iron differed at each heating temperature. However, results from the analyses of variance for PV (Appendix Table 6) showed that no interactions ( $p > 0.05$ ) existed between the heating temperature and the type of iron added in the beef model system I. A possible explanation for this difference between PV and TBA values might be that the PV method was less accurate and thus had a less sensitive statistical analysis due to its large measurement errors (Appendix Tables 5 and 6).

The TBA and PV values for the effects of heat and various forms of iron on lipid oxidation in the emulsions of beef intramuscular fat (model system II) are summarized in Tables 5.7 and 5.8, respectively. A Pearson correlation coefficient of 0.79 was found between TBA and PV values, suggesting that the catalytic abilities of iron fractions on the formation of primary and secondary oxidative products were similar.

The extent of lipid oxidation in the raw control sample (no heat and no iron added beef emulsion) was found to be lower ( $p < 0.05$ ) than that in the heat and/or iron treated fat emulsions, with the exception of ferritin,  $\text{FeCl}_2$ , and  $\text{FeCl}_3$  treated fat emulsions at room temperature (Tables 5.7 and 5.8). The results suggested the treatment of heat, some of

**Table 5.7** Effect of heat and various iron fractions on lipid oxidation expressed as TBA values<sup>1,2</sup> in emulsion of beef intramuscular fat (model system II) stored at 4°C for 48h.

Iron Treatment	Temperature				
	Raw	55°C	70°C	85°C	100°C
Control	0.84 <sup>a</sup>	0.94 <sup>b</sup>	1.23 <sup>c</sup>	1.33 <sup>d</sup>	1.40 <sup>dc</sup>
H <sub>2</sub> O Extractable Fe	1.56 <sup>f</sup>	1.75 <sup>g</sup>	2.06 <sup>h</sup>	2.34 <sup>a</sup>	2.43 <sup>p</sup>
Diffusate Fe	0.93 <sup>b</sup>	1.05 <sup>c</sup>	1.32 <sup>d</sup>	1.43 <sup>i</sup>	1.46 <sup>ci</sup>
Non-diffusate Fe	1.45 <sup>c</sup>	1.66 <sup>f</sup>	1.98 <sup>g</sup>	2.31 <sup>m</sup>	2.37 <sup>mop</sup>
Hematin	1.08 <sup>c</sup>	1.24 <sup>d</sup>	1.50 <sup>e</sup>	1.60 <sup>j</sup>	1.69 <sup>j</sup>
Hemoglobin	1.32 <sup>d</sup>	1.48 <sup>e</sup>	1.86 <sup>f</sup>	2.19 <sup>k</sup>	2.22 <sup>kn</sup>
Myoglobin	1.32 <sup>d</sup>	1.45 <sup>e</sup>	1.88 <sup>f</sup>	2.20 <sup>kl</sup>	2.29 <sup>lno</sup>
Ferritin	0.85 <sup>a</sup>	0.94 <sup>b</sup>	1.24 <sup>c</sup>	1.33 <sup>dh</sup>	1.40 <sup>ch</sup>
FeCl <sub>2</sub>	0.83 <sup>a</sup>	0.95 <sup>b</sup>	1.25 <sup>c</sup>	1.34 <sup>de</sup>	1.40 <sup>eg</sup>
FeCl <sub>3</sub>	0.84 <sup>a</sup>	0.93 <sup>b</sup>	1.23 <sup>c</sup>	1.33 <sup>df</sup>	1.39 <sup>cf</sup>

<sup>1</sup>Each TBA value (mg malondialdehyde/kg sample) is the least-squares means of four measurements.

<sup>2</sup>Standard error of mean = 0.034.

<sup>abcde fghijklmnop</sup>Means in the same row or column not bearing a common superscript letter are different (p<0.05).

iron forms, or the combination of both could promote lipid oxidation in the model system. TBA and PV values in the beef model system II catalyzed by the same type of iron continuously increased as the temperature increased up to 100°C. The greatest increase rate in lipid oxidation in the beef fat emulsion with catalytic iron was found between temperatures of 55°C and 85°C. This was also the temperature range that most hemoproteins might be denatured (Chapter IV).

**Table 5.8** Effect of heat and various iron fractions on lipid oxidation expressed as PV<sup>1,2</sup> in emulsion of beef intramuscular fat (model system II) stored at 4°C for 48h.

Iron Treatment	Temperature				
	Raw	55°C	70°C	85°C	100°C
Control	2.51 <sup>a</sup>	4.84 <sup>b</sup>	8.15 <sup>c</sup>	9.70 <sup>cd</sup>	10.65 <sup>de</sup>
H <sub>2</sub> O Soluble Fe	6.21 <sup>f</sup>	9.12 <sup>i</sup>	12.42 <sup>km</sup>	13.80 <sup>mo</sup>	14.24 <sup>mp</sup>
Diffusate Fe	5.10 <sup>bf</sup>	6.93 <sup>g</sup>	9.41 <sup>ci</sup>	10.59 <sup>din</sup>	10.86 <sup>ci</sup>
Non-diffusate Fe	6.14 <sup>f</sup>	8.50 <sup>hi</sup>	12.25 <sup>kl</sup>	13.35 <sup>lo</sup>	13.52 <sup>lp</sup>
Hematin	4.57 <sup>b</sup>	7.06 <sup>gh</sup>	10.04 <sup>ij</sup>	11.07 <sup>ja</sup>	11.43 <sup>ej</sup>
Hemoglobin	5.70 <sup>bf</sup>	7.90 <sup>ghi</sup>	11.73 <sup>jk</sup>	13.14 <sup>ko</sup>	13.47 <sup>kp</sup>
Myoglobin	5.61 <sup>bf</sup>	7.78 <sup>ghi</sup>	11.73 <sup>jk</sup>	13.11 <sup>ko</sup>	13.32 <sup>kp</sup>
Ferritin	2.64 <sup>a</sup>	4.86 <sup>b</sup>	8.21 <sup>cf</sup>	9.77 <sup>df</sup>	10.60 <sup>ef</sup>
FeCl <sub>2</sub>	3.08 <sup>a</sup>	4.98 <sup>b</sup>	8.31 <sup>ch</sup>	9.84 <sup>dh</sup>	10.98 <sup>eh</sup>
FeCl <sub>3</sub>	2.92 <sup>a</sup>	4.99 <sup>b</sup>	8.30 <sup>cg</sup>	9.90 <sup>dg</sup>	10.99 <sup>eg</sup>

<sup>1</sup>Each PV (milliequivalents of peroxide per 1000 gm. of sample) is the least-squares means of four measurements.

<sup>2</sup>Standard error of mean = 0.68.

<sup>abcde fghijklmnop</sup> Means in the same row or column not bearing a common superscript letter are different (p<0.05).

The unfolding of the hemoproteins due to the heat denaturation could cause more exposure of heme to the unsaturated fatty acids which would result in greater catalytic activity (Eriksson et al., 1971; Greene, 1975).

The results indicated that water-extractable iron or non-diffusate iron had the highest catalytic ability in both raw and cooked beef fat emulsions, followed by the Hb and Mb fractions, and hematin (Tables 5.7 and 5.8). However,



diffusate iron had little catalytic effect on the oxidation of lipid in beef model system II. Ferritin fraction,  $\text{Fe}^{2+}$ , and  $\text{Fe}^{3+}$  did not have any effects on lipid oxidation in the model system II compared to the control samples at each temperature.

Orthophosphate buffer and beef intramuscular fat were the components of model system II used in this experiment. Compared to polyphosphates, such as STPP (sodium tripolyphosphate) and TSPP (tetrasodium pyrophosphate), the chelating ability of orthophosphate with free iron was relatively weak (Molins, 1991). However, chelating ability of the orthophosphate might be strong enough to bind the small amount of iron in model system II. Furthermore, ferric and ferrous orthophosphate are insoluble in water at neutral pH (Inorganic Chemistry Edit. Group, 1978). Chelation of free iron by orthophosphate and/or the formation of insoluble salts between free iron ( $\text{Fe}^{2+}$  or  $\text{Fe}^{3+}$ ) and orthophosphate might be the reasons why diffusate iron had a weak catalytic effect and the ferritin fraction,  $\text{Fe}^{2+}$ , and  $\text{Fe}^{3+}$  had no effect on lipid oxidation in model system II.

The water-extractable iron fraction included fractions of diffusate and non-diffusate iron. The non-diffusate iron fraction contained mainly hemoproteins (Mb and Hb) and a small amount of ferritin. Both water-extractable and non-diffusate iron fractions had strong catalytic ability on lipid oxidation. But diffusate iron had a weak catalytic

ability on lipid oxidation, and ferritin fraction had no catalytic effect. Therefore, heme iron of the hemoprotein in the water-extractable and the non-diffusate fractions was probably responsible for the high catalytic activity on lipid oxidation in model system II.

Results from the analyses of variance (Appendix Table 5 and 6) for TBA or PV in chicken model system II indicated that both iron and heat affected ( $p < 0.05$ ) lipid oxidation in the model system. An interaction ( $p < 0.05$ ) existed between the heating temperature and the type of iron added for the chicken model system II on the TBA values (Appendix Table 5). Such interaction suggested that heat had different effect on lipid oxidation by various iron fractions in the chicken model system II. However, results from the analyses of variance for PV (Appendix Table 6) showed no such interaction ( $p > 0.05$ ) for the chicken model system II. Possible explanation for such difference might be that the PV method was less accurate and thus the effect became less sensitive in the statistical analysis due to its large measurement errors (Appendix Tables 5 and 6).

TBA and PV values for the effects of heat and various forms of iron on lipid oxidation in chicken model system II are summarized in Tables 5.9 and Table 5.10, respectively. TBA values correlated well with PV in chicken model system II with a Pearson correlation coefficient 0.85. The extent of lipid oxidation in the raw control sample (no heat and no

**Table 5.9** Effect of heat and various iron fractions on lipid oxidation expressed as TBA values<sup>1,2</sup> in emulsion of chicken intramuscular fat (model system II) stored at 4°C for 48h.

Iron Treatment	Temperature				
	Raw	55°C	70°C	85°C	100°C
Control	0.97 <sup>a</sup>	1.14 <sup>ab</sup>	1.33 <sup>bc</sup>	1.43 <sup>cd</sup>	1.43 <sup>ce</sup>
H <sub>2</sub> O Soluble Fe	1.88 <sup>j</sup>	2.15 <sup>l</sup>	2.83 <sup>o</sup>	3.40 <sup>qr</sup>	3.52 <sup>rt</sup>
Diffusate Fe	1.17 <sup>ai</sup>	1.41 <sup>k</sup>	1.62 <sup>k</sup>	1.85 <sup>m</sup>	1.92 <sup>m</sup>
Non-diffusate Fe	1.78 <sup>j</sup>	2.07 <sup>l</sup>	2.69 <sup>no</sup>	3.28 <sup>oq</sup>	3.40 <sup>qt</sup>
Hematin	1.30 <sup>i</sup>	1.55 <sup>k</sup>	1.95 <sup>l</sup>	2.10 <sup>ln</sup>	2.17 <sup>n</sup>
Hemoglobin	1.70 <sup>j</sup>	1.94 <sup>l</sup>	2.57 <sup>mn</sup>	3.11 <sup>o</sup>	3.24 <sup>os</sup>
Myoglobin	1.70 <sup>j</sup>	1.95 <sup>l</sup>	2.46 <sup>m</sup>	3.13 <sup>op</sup>	3.27 <sup>ps</sup>
Ferritin	0.98 <sup>af</sup>	1.13 <sup>bf</sup>	1.35 <sup>ch</sup>	1.45 <sup>dh</sup>	1.48 <sup>ch</sup>
FeCl <sub>2</sub>	0.99 <sup>ah</sup>	1.19 <sup>bhj</sup>	1.34 <sup>cgi</sup>	1.44 <sup>dg</sup>	1.48 <sup>cg</sup>
FeCl <sub>3</sub>	0.98 <sup>ag</sup>	1.15 <sup>bgi</sup>	1.31 <sup>cfi</sup>	1.46 <sup>df</sup>	1.49 <sup>cf</sup>

<sup>1</sup>Each TBA value (mg malondialdehyde/kg sample) is the least-squares means of four measurements.

<sup>2</sup>Standard error of mean = 0.07.

Means in the same row or column not bearing a common superscript letter are different (p<0.05).

iron added chicken fat emulsion) was lower (p<0.05) than that in the heat and iron treated fat emulsion, except in the ferritin, FeCl<sub>2</sub>, or FeCl<sub>3</sub>-treated fat emulsions at room temperatures (Tables 5.9 and 5.10). Heat promoted lipid oxidation in the chicken model system II with or without added iron fractions. TBA and PV in the chicken model system II catalyzed by the same type of iron continuously increased as the temperature increased up to 100°C. The greatest

increase rate in lipid oxidation in the chicken fat emulsion with catalytic iron was found between temperatures of 55°C and 85°C.

**Table 5.10** Effect of heat and various iron fractions on lipid oxidation expressed as PV<sup>1,2</sup> in emulsion of chicken intramuscular fat (model system II) stored at 4°C for 48h.

Iron Treatment	Temperature				
	Raw	55°C	70°C	85°C	100°C
Control	4.55 <sup>a</sup>	7.79 <sup>b</sup>	9.10 <sup>bc</sup>	9.92 <sup>bd</sup>	10.69 <sup>bc</sup>
H <sub>2</sub> O Soluble Fe	11.90 <sup>kn</sup>	13.20 <sup>nr</sup>	17.54 <sup>sw</sup>	19.89 <sup>w</sup>	20.27 <sup>w</sup>
Diffusate Fe	6.17 <sup>ai</sup>	9.81 <sup>bq</sup>	12.65 <sup>qr</sup>	14.47 <sup>r</sup>	14.92 <sup>r</sup>
Non-diffusate Fe	10.52 <sup>ikm</sup>	12.22 <sup>mqr</sup>	16.07 <sup>sv</sup>	18.99 <sup>v</sup>	19.28 <sup>v</sup>
Hematin	7.97 <sup>ij</sup>	11.11 <sup>jq</sup>	14.99 <sup>s</sup>	15.46 <sup>s</sup>	16.00 <sup>s</sup>
Hemoglobin	9.54 <sup>kl</sup>	11.20 <sup>lqr</sup>	15.65 <sup>su</sup>	17.78 <sup>u</sup>	18.31 <sup>u</sup>
Myoglobin	9.42 <sup>jk</sup>	11.23 <sup>kqr</sup>	15.33 <sup>st</sup>	17.54 <sup>t</sup>	18.09 <sup>t</sup>
Ferritin	4.62 <sup>af</sup>	7.69 <sup>bfi</sup>	9.27 <sup>ci</sup>	9.93 <sup>di</sup>	10.77 <sup>ci</sup>
FeCl <sub>2</sub>	4.72 <sup>ag</sup>	7.79 <sup>bgo</sup>	9.43 <sup>co</sup>	10.24 <sup>do</sup>	10.69 <sup>co</sup>
FeCl <sub>3</sub>	4.84 <sup>ah</sup>	7.86 <sup>bhp</sup>	9.37 <sup>cp</sup>	9.97 <sup>dp</sup>	10.44 <sup>cp</sup>

<sup>1</sup>Each PV (milliequivalents of peroxide per 1000 gm. of sample) is the least-squares means of four measurements.

<sup>2</sup>Standard error of mean = 1.23.

Means in the same row or column not bearing a common superscript letter are different (p<0.05).

The catalytic ability of various forms of iron on lipid oxidation in chicken model system II could be ranked in the following order: water-extractable iron > non-diffusate iron > Hb ≈ Mb > hematin > diffusate iron > ferritin ≈ FeCl<sub>2</sub> ≈

FeCl<sub>3</sub> (Tables 5.9 and 5.10). Diffusate iron was found to have little catalytic effect on lipid oxidation in chicken model system II. No catalytic effect ( $p > 0.05$ ) on lipid oxidation was found by adding ferritin, Fe<sup>2+</sup>, or Fe<sup>3+</sup> in the chicken model system II. Chelation of free iron by orthophosphate and/or the formation of insoluble salt between free iron (Fe<sup>2+</sup> or Fe<sup>3+</sup>) and orthophosphate might be the reasons why diffusate iron had a weak catalytic effect and ferritin, Fe<sup>2+</sup>, and Fe<sup>3+</sup> fractions had no effect on lipid oxidation in model system II.

To compare the results with beef and chicken model system II, the same order was found in the catalytic ability of various types of iron on lipid oxidation. However, TBA and PV values in the emulsion of chicken fat appeared to be slightly higher than those in the corresponding iron-catalyzed beef fat emulsion. No of statistical analyses were made to compare TBA or PV values between the beef and chicken model systems because of the violation in the assumptions for such an analysis (different error terms were calculated between the two model systems). The higher content of unsaturated fatty acids, especially polyunsaturated fatty acids (Table 5.2), in the chicken model system II than in beef was the possible explanation for the difference in lipid oxidation between beef and chicken.

In comparing results of model systems I and II, similar degrees of lipid oxidation were found when iron catalysts

were not added in beef or chicken model system I and II. However, when a iron catalyst was added in the model systems, the TBA and PV in model system I appeared to be higher than the corresponding values in model system II. This might suggest that the catalytic ability of iron in the meat residue was higher than the one in the fat emulsion. Model system I contained water-insoluble proteins, muscle fiber as well as lipids, while model system II only contained beef or chicken fat and orthophosphate buffer. Polyphosphates, such as TSPP, STPP, and SPG (sodium polyphosphate glass), have been found to have antioxidant properties in meats, while mono- and disodium orthophosphates were ineffective in preventing lipid oxidation (Tims and Watts, 1958; Shahidi et al., 1986). The higher TBA or PV in iron-catalyzed model system I samples meant that iron had higher catalytic ability for lipid oxidation in muscle residues than in fat emulsions. A possible explanation for the results might be that some compounds such as proteins in muscle residues had a synergistic effect with iron to catalyze lipid oxidation.

Molins et al. (1987) reported that polyphosphate STPP and TSPP interfered with the TBA distillation assay resulting in lower TBA values. However, in the current experiment, the PV in fat emulsions were also lower than corresponding value in meat residues. Therefore, it was unlikely that the phosphates used in model system II interfered with the TBA distillation assay.

The water-extracted meat residue (model system I) was considered better for the study of catalysis of lipid oxidation than fat emulsions (model system II) because it was more similar to muscle and it had smaller coefficient of variation (C.V.) for both TBA and PV compared with the corresponding C.V. in beef or chicken fat emulsion. The smaller C.V. in model system I meant that measurement of lipid oxidation in meat residues was more consistent and had less variations among the samples than in fat emulsions.

From the present results, it may be concluded that all of the various forms of iron present in meat contributed to the overall development of lipid oxidation in meat during heating. The order of the catalytic ability of various forms of iron on lipid oxidation in raw meat might be as follows: diffusate iron > hemoglobin and myoglobin > hematin > ferritin > water insoluble iron. The order for oxidation catalysis in cooked meat might be as follows: diffusate iron > hemoglobin  $\approx$  myoglobin > ferritin > hematin > water insoluble iron. The degree of lipid oxidation on cooked meat was related to the intensity of the heat treatment. Beef model systems were found to be less susceptible to lipid oxidation than chicken model systems, probably due to the increased amounts of unsaturated fatty acid content in chicken fat.

## **CHAPTER VI**

### **SUMMARY**



The results from the first experiment indicated that heme pigments (Hb and Mb) in beef and chicken muscles were separated from other water soluble proteins, but not from one another, by size exclusion HPLC (SE-HPLC). Standard Mb was used to quantify the content of total pigments in beef and chicken muscles. The lowest amount of total pigments determined by SE-HPLC was  $0.001 \mu\text{g}/\mu\text{l}$  compared to  $0.037 \mu\text{g}/\mu\text{l}$  with spectrophotometric analysis. The Mb and Hb pigments in bovine muscles, but not in chicken muscle were completely separated and accurately determined with hydrophobic interaction HPLC (HIC) due to different degrees of hydrophobic interaction with the n-butyl groups contained in the column and different solubilities in the salt buffers. The retention times of Mb, and Hb were 11.40, and 15.70 minutes, respectively. The minimum detectable levels for Mb and Hb were  $0.085$  and  $0.186 \mu\text{g}/\mu\text{l}$ , respectively, with HIC.

Results of analyses of variance indicated that the contents of total pigments, Hb, and Mb were variable ( $p > 0.05$ ) with individual beef animal. The concentrations of total pigments of beef longissimus and psoas major determined by SE-HPLC were  $4.96 \pm 0.19$ , and  $4.72 \pm 0.18 \text{ mg/g}$  sample, respectively, for beef psoas major. The concentrations of Hb and Mb in beef longissimus measured by HIC were  $0.82 \pm 0.07$  and  $3.44 \pm 0.43 \text{ mg/g}$  sample, respectively, and that of  $0.92 \pm 0.08$  and  $3.60 \pm 0.3 \text{ mg/g}$  sample, respectively, for beef psoas major.

The SE method had advantages of high sensitivity and measurement consistency and, therefore, is recommended for determination of total pigments in muscles, especially for those species with low Mb content such as poultry. The HIC method was convenient in separating Mb and Hb in beef muscle but less sensitive than SE or spectrophotometric techniques.

The effect of heat on the distribution of iron in six fractions of water-soluble, water-insoluble, diffusate, hematin, total heme, and ferritin in beef and chicken muscles were determined in the second experiment. Heat had significant effect on the distribution of iron in various fractions in both bovine longissimus and chicken thigh muscles. Iron contents in various fractions in beef and chicken muscles followed different patterns of change as the heating temperature increased. Iron contents decreased in water-soluble fractions and increased in water-insoluble fractions as temperature increased to 100°C. The greatest change of iron contents in the water-soluble and insoluble fractions was found at the heating temperature of 70°C. The content of heme iron was decreased more from 55°C to 85°C than from 27°C to 55°C or 85°C to 100°C. Oxidative cleavage of the porphyrin ring might not be the only way to cause the decrease of heme iron because the amount of the increased diffusate iron was smaller than the amount of decreased heme iron at each temperature level. When the heating temperature reached 85°C, hematin content in beef samples began to

increase from the control sample. Hematin content had a significant increase in both beef and chicken samples when the heating temperature reached 100°C. The content of ferritin iron decreased as the temperature increased. Significant decrease of ferritin iron in beef and chicken was found when the temperature was increased from 55°C to 70°C. The denaturation of ferritin at high temperatures resulted in insolubility in water, and so the contents of ferritin iron in meat samples heated to 85°C and 100°C were unable to be determined by the chromatographic technique.

Two types of model systems were used to study the catalytic abilities of nine forms of iron (water-extractable iron, diffusate iron, non-diffusate iron, hematin,  $\text{FeCl}_2$ ,  $\text{FeCl}_3$ , Hb, Mb, and ferritin fractions) on lipid oxidation in the third experiment. Model system I were the water-extracted beef or chicken residues. Results from analyses of variance of TBA and PV indicated that lipid oxidation in the beef and chicken residues was affected ( $p < 0.05$ ) by heat and the type of iron added. Interactions ( $p < 0.05$ ) existed between temperature and the type of iron added in the meat residues, which suggested that heat had different effect on lipid oxidation in model system I catalyzed by various forms of iron. The treatment of heat, irons, or the combination of both could promote lipid oxidation in model system I. TBA and PV in model system I containing the same type of added iron increased as the heating temperature increased to 85°C.

Lipid oxidation in the beef residue catalyzed by  $\text{FeCl}_2$ ,  $\text{FeCl}_3$ , ferritin fraction, or hematin continued to increase as temperature increased from 85°C to 100°C. However, lipid oxidation for either the beef residues containing the other iron fractions or the chicken residues containing any of the iron fractions was decreased, when the heating temperature increased from 85°C to 100°C. The catalytic ability of different forms of iron on lipid oxidation in raw beef and chicken residues could be ranked as  $\text{Fe}^{2+}$  > water extractable iron > diffusate iron > non-diffusate > Hb fraction and Mb fraction > hematin >  $\text{Fe}^{3+}$  and ferritin. The order of the catalytic ability of different forms of iron on lipid oxidation in cooked beef and chicken residues might be  $\text{Fe}^{2+}$  > water extractable iron > diffusate iron > non-diffusate > Hb fraction and Mb fraction >  $\text{Fe}^{3+}$ , ferritin, and hematin. Chicken model systems appeared to have higher degrees of lipid oxidation and had higher contents of linolenic acid than the corresponding beef model systems.

Model system II used in the experiment was the emulsion of beef or chicken intramuscular fat. Heat treatment and type of iron both affected ( $P < 0.05$ ) lipid oxidation in the beef and chicken model system II. TBA and PV values in the beef and chicken model system II catalyzed by the same type of iron were increased ( $p < 0.05$ ) as the heating temperature increased. The greatest increase rate of lipid oxidation was at the temperature range from 55°C and 85°C. Water

extractable iron had the highest catalytic activity in both raw and chicken fat emulsion (model system II), followed by non-diffusate iron, Hb and Mb fractions, and hematin. Diffusate iron had weak catalytic ability on lipid oxidation, while ferritin fraction,  $\text{FeCl}_2$ , and  $\text{FeCl}_3$  did not affect lipid oxidation in the model system II. Chelation of free iron by orthophosphate and/or the formation of insoluble salts between free iron ( $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$ ) and orthophosphate might be the reasons why diffusate iron had a weak catalytic effect and ferritin fraction,  $\text{Fe}^{2+}$ , and  $\text{Fe}^{3+}$  had no effect on lipid oxidation in model system II. Heme iron in the water extractable and non-diffusate fractions was probably responsible for the high catalytic activity on lipid oxidation in the model system II. Because model system I was more similar to muscle and provided more consistent TBA and PV values than model system II, it was considered a more suitable model system to study catalysis of lipid oxidation.

## **CHAPTER VII**

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## **APPENDICES**

### Appendix Method 1

#### Procedure of digesting meat sample for total iron determination by atomic absorption spectrophotometry

About 10 grams of meat slurry were weighed into a 250-ml beaker and 40 ml of nitric acid and perchloric acid digesting solution (7:1 v:v) were added. The samples were soaked in the digesting solution at room temperature for at least 16 hours with watch glass covers on the beakers. After the soaking period, the samples were boiled on a temperature-controlled hot plate (Corning, Model PC-520, Houston, TX) until they turned from red/brown color to dark residues. At this point, the beakers were removed from the hot plate and allowed to cool to room temperature. About 20 ml of the digesting solution was then added to the beakers. The heating and cooling processes were repeated once or twice until a clear light yellow or colorless solution remained at the bottom of the beaker. The content in the beaker was transferred to a 50-ml volumetric flask. The beaker was washed three times with deionized water. Each wash was added to the volumetric flask and made to volume with deionized water. The samples were then transferred to 20-ml glass scintillation vials, capped and kept at room temperature until atomic absorption spectrophotometric analysis. The atomic absorption spectrophotometric analysis was performed at a wavelength of 248.3 nm and a slit width of 0.2 nm with an air-acetylene oxidizing flame (Model 3030B, Perkin Elmer

Corp., Norwalk, CT). Standard solutions of 0.5, 1.5, 2.5, 3.5, and 5 ppm were prepared by appropriately diluting a certified atomic absorption standard iron reference solution (1000 ppm) (Sigma Chemical Co., St Louis, MO). This procedure was described by Guzman (1987).

## Appendix Method 2

### Method for the isolation and purification of total lipids from animal tissues

(a) Extraction of lipids. A mixture of chloroform and methanol (2:1 by volume) was used as solvent to extract fat from meat. Both chloroform and methanol were purified by steam distillation. The desired amount of ground meat was homogenized with a 17-fold volume of solvent mixture for 3 minutes. The homogenate was then filtered through a No. 1 Whatman filter paper into a glass-stopped vessel.

(b) Washing of Extract. The filtered extract was washed by mixing thoroughly with the upper phase of a pure solvent containing 0.02%  $\text{CaCl}_2$  to remove non-lipid contaminants. (The upper phase of a pure solvent was prepared by mixing chloroform, methanol, and 0.04% aqueous  $\text{CaCl}_2$  in the proportions 8:4:3 by volume. When the mixture was allowed to stand, a biphasic system was obtained. The two phases were collected separately and stored in glass bottles. The approximate proportions of chloroform, methanol, and water in the upper phase were 3:48:47 by volume). The resulting mixtures of meat extract and solvent were separated into two phases by standing. The upper phase was removed as much as possible by siphoning and the lower phase was the total pure fat extract.

(c) Removal of solvents. After separating the upper phase of the extract from the lower phase which contained the

total pure intramuscular fat, the solvent in the lower phase was removed from the extracted fat by drying under vacuum. The purified fat was kept at  $-18^{\circ}\text{C}$  and was ready to be used in experiment. This method was described by Folch et al. (1957).

### Appendix Method 3

#### TBA (Thiobarbituric acid) test

##### Reagents

1. TBA reagents. 0.02 M 2-thiobarbituric acid in 90% glacial acetic acid. 2.88 g of 2-thiobarbituric acid is brought to one liter with 90% glacial acetic acid (900 ml acid + 100 ml deionized water).
2. TMP standard. 0.165 ml 1, 1, 3, 3-tertramethoxypropane in 200 ml deionized water and 1 ml of 1 N HCL. Put in 50°C water bath for one hour using a stoppered Erlenmeyer flask. Dilute this to 1000 ml. This solution can be stored in the at 4°C for three days and diluted as needed.
3. Working TMP standard. 10 ml TMP standard is added to 500 ml distilled water.
4. 4 N HCL solution.

##### Method

1. Weigh 10 g of sample into a 800 ml kjeldahl flask.
2. Add 97.5 ml deionized water into the flask.
3. Add 2.5 ml of 4 N HCL.
4. Add 5 drops of Dow antifoam A.
5. Add 5 glass beads.
6. Heat the flask on the distillation apparatus.
7. Collect 50 ml of the distillate in a centrifuge tube.
8. Mix the distillate by shaking the tube 5 times.

9. Pipette 5 ml of the distillate into a 20 ml test tube (use 5 ml of deionized water for the blank).
- 10 Add 5 ml of TBA reagent to each tube.
11. Mix the contents by vortexing the tube at medium speed for 5 seconds.
12. Stopper the tube with rubber stopper.
13. Place the tube in a boiling water bath for 35 minutes.
- 14 Cool the tube in tap water for 10 minutes.
15. Transfer the solution into a cuvette and measure the optical density (O.D.) against the blank to obtain 100% transmission with a spectrophotometer (Model u-200 uv/vis, spectrophotometer, Danbury, CT) at a wavelength of 535 nm.

#### Determination of Standard Curve

1. Prepare working TMP standard solution. The concentration of the working TMP standard solution was  $2 \times 10^{-5}$  M.
2. Make the non-distilled standards according to the following table and continue the procedures from steps 11-15.

Tube No.	TMP Working Standard (ml)	Deionized Water (ml)	TBA Reagent (ml)	$\mu$ mole MA
1	0.5	4.5	5	0.01
2	1.0	4.0	5	0.02
3	1.5	3.5	5	0.03
4	2.0	3.0	5	0.04
5	2.5	2.5	5	0.05



3. Plot optical density vs.  $\mu$ moles of MA to obtain standard curve.

#### Determination of Recovery

1. Make distilled standards from the working TMP standard solution according to the following table and continue the process from steps 4-15.

Flask No.	TMP Working Standard (ml)	4 N HCL (ml)	Deionized Water (ml)	$\mu$ mole MA
1	5.0	2.5	92.5	0.01
2	10.0	2.5	87.5	0.02
3	15.0	2.5	82.5	0.03
4	20.0	2.5	77.5	0.04
5	25.0	2.5	72.5	0.05

2. The percentage of recovery of malondialdehyde (MA) in distillation was calculated by dividing the O.D. of the distilled standard over O.D. of nondistilled standard. The average recovery value 64% was used to calculate TBA members.

#### Calculation of TBA number

1. The TBA number is expressed as mg of malondialdehyde per 1000 g of sample and is calculated by multiplying the absorbance by a constant K. The K value is obtained from the standard curve and the known dilutions shown as follows:

$$K \text{ (Extraction)} = \frac{S}{A} \times MW \times \frac{10}{W} \times \frac{100}{P}$$

where S = Standard concentration ( $1 \times 10$  moles 1, 1, 3,  
3-tetraethoxypropane / 5 ml)

A = Absorbance of standard

MW = Molecular weight of malondialdehyde = 72

W = Weight of sample

P = Percent recovery

2. TBA number =  $K \times$  optical density of sample

(This method was described by Tarladgis et al., 1960)

**Appendix Method 4**  
**Peroxide value determination**

**Reagents**

1. Chloroform, reagent grade.
2. Sodium sulfate, anhydrous.
3. Starch solution 1%, made fresh daily.
4. Potassium iodine, saturated, 140 g per 100 ml water, made fresh daily and stored in brown bottle.
5. Sodium thiosulfate, 0.1 N from which 0.01 N  $\text{Na}_2\text{S}_2\text{O}_3$  is made fresh daily.
6. Acetic acid, glacial.

**Method**

1. Weigh 5 g sample.
2. Pre-condition blender jar with chloroform by adding about 100 ml and turning blender on a few seconds, discard chloroform.
3. Transfer the sample to the blender jar.
4. Add 1/4 teaspoon of anhydrous  $\text{Na}_2\text{SO}_4$  and 28.7 ml chloroform.
5. Blend in a Waring blender for 2 minutes.
6. Filter the mix through No. 12 Whatman fluted filter paper into a 500 ml Erlenmeyer flask.
7. Add 30 ml of glacial acetic acid and 2 ml of saturated potassium iodine to the chloroform extract and let it react for exactly 2 minutes with occasional swirling.

8. After exactly 2 minutes add 100 ml of deionized water to stop the reaction.
9. Add 2 ml of starch and titrate immediately with 0.1 N sodium thiosulfate.

Peroxide value as milliequivalents of peroxide per 1000

$$\text{g of sample} = \frac{S \times N \times 1000}{\text{weight of sample}}$$

Where S = Titration of sample (ml)

N = Normality of sodium thiosulfate solution

(This method was described by Koniecko, 1985)

### **Appendix Method 5**

#### **Method for preparation of fatty acid methyl esters**

Approximately 150 mg of fatty material was added to a 50-ml volumetric flask. Four milliliters of 0.5 N methanolic sodium hydroxide was added to the mixture which was heated on a steam bath until the fat globules went into solution. This step would take about five minutes. Five milliliters of boron trifluoride ( $\text{BF}_3$ )-methanol (Sigma Chemical Co., St. Louis, MO) was added to the flask and the mixture was boiled for 2 minutes. Enough of a saturated sodium chloride solution (about 40 ml) was added to the flask to float the methyl esters up into the narrow neck of the flask for withdrawal with a syringe. The methyl esters were ready for gas chromatographic analysis of fatty acid composition. This method was described by Metcalfe et al., 1966.

Appendix Table 1

Analysis of variance for the contents of total pigments in beef and chicken muscles.

Source	Beef			Chicken		
	df <sup>a</sup>	SS <sup>b</sup>	p>F	df <sup>a</sup>	SS <sup>b</sup>	p>F
Animal	4	12.005	0.0001	2	0.003	0.0081
Muscle	1	0.002	0.9164	1	0.042	0.0001
Animal*Muscle	4	3.335	0.0016	2	0.009	0.0001
Method	2	2.126	0.0031	1	0.001	0.0666
Muscle*Method	2	0.633	0.1523	1	0.001	0.5525
Error	46	7.431		16	0.004	
Corrected Total	59			23		

<sup>a</sup>df = degrees of freedom.

<sup>b</sup>SS = sums of squares for type III estimable function.

Appendix Table 2

Analysis of variance for the contents of hemoglobin and myoglobin in beef muscles.

Source	df <sup>a</sup>	SS <sup>b</sup>	p>F
Cattle	4	8.578	0.0002
Muscle	1	0.134	0.3210
Muscle*Cattle	4	2.728	0.0129
Error	10	0.320	
Corrected Total	19		

<sup>a</sup>df = degrees of freedom.

<sup>b</sup>SS = sums of squares for type III estimable function.

Appendix Table 3

Analysis of variance for the iron contents in various fractions of bovine longissimus.

Source	df <sup>a</sup>	TFE <sup>b</sup>	WSFE <sup>b</sup>	WIFE <sup>b</sup>	DIAFE <sup>b</sup>	HE <sup>b</sup>	THEME <sup>b</sup>	df <sup>c</sup>	FER <sup>b</sup>
Replicate	1	0.8040 <sup>d</sup> (0.6567) <sup>e</sup>	1.04 (0.6135)	7.12 (0.3174)	0.0005 (0.9065)	0.0005 (0.5969)	0.0461 (0.4653)	1	0.0008 (0.6089)
Temp	4	0.3524 (0.9989)	429.80 (0.0001)	365.95 (0.0001)	4.03 (0.0001)	0.0288 (0.0191)	25.05 (0.0001)	2	0.0777 (0.0029)
Error	14	54.58	54.55	92.68	0.4895	0.0239	1.15	8	0.0235

<sup>a</sup>df = degrees of freedom.

<sup>b</sup>TFE = total iron; WSFE = iron in water soluble fraction; WIFE = iron in water insoluble fraction; DIAFE = diffusate iron; HE = hematin iron; THEME = total heme iron; FER = ferritin iron.

<sup>c</sup>df = degrees of freedom for ferritin because iron levels in sample were not detectable at 85°C and 100°C.

<sup>d</sup>Sums of squares for type III estimable function.

<sup>e</sup>The value  $p > F$ .



Appendix Table 4

Analysis of variance for the iron contents in various fractions of chicken thigh muscles.

Source	df <sup>a</sup>	TFE <sup>b</sup>	WSFE <sup>b</sup>	WIFE <sup>b</sup>	DIAFE <sup>b</sup>	HE <sup>b</sup>	THEME <sup>b</sup>	df <sup>c</sup>	FER <sup>b</sup>
Replicate 1		0.5578 <sup>d</sup> (0.5992) <sup>cd</sup>	0.0720 (0.5696)	5.97 (0.2325)	0.0328 (0.3521)	0.0003 (0.6930)	0.0259 (0.1311)	1	0.0001 (0.7852)
Temp	4	0.5017 (0.9913)	26.80 (0.0001)	31.94 (0.1376)	0.7440 (0.0085)	0.0655 (0.0012)	1.02 (0.0001)	2	0.1482 (0.0001)
Error	14	27.01	2.97	53.68	0.4956	0.0276	0.1411	8	0.0076

<sup>a</sup>df = degrees of freedom.

<sup>b</sup>TFE = total iron; WSFE = iron in water soluble fraction; WIFE = iron in water insoluble fraction; DIAFE = diffusate iron; HE = hematin iron; THEME = total heme iron; FER = ferritin iron.

<sup>c</sup>df = degrees of freedom for ferritin because iron levels in sample were not detectable at 85°C and 100°C.

<sup>d</sup>Sums of squares for type III estimable function.

<sup>e</sup>The value p>F.

Appendix Table 5

Analysis of variance for TBA values in beef and chicken model systems I and II<sup>a</sup>.

Source	df <sup>b</sup>	Beef				Chicken			
		MSI <sup>a</sup>		MSII <sup>a</sup>		MSI <sup>a</sup>		MSII <sup>a</sup>	
		SS <sup>c</sup>	P>F	SS <sup>c</sup>	P>F	SS <sup>c</sup>	P>F	SS <sup>c</sup>	P>F
Iron	9	263.07	0.0001	25.06	0.0001	549.60	0.0001	73.56	0.0001
Rep(Iron)	10	0.02	0.9986	0.04	0.3573	0.19	0.8647	0.03	0.9993
Temp	4	88.31	0.0001	15.19	0.0001	570.28	0.0001	29.90	0.0001
Temp*Iron	36	14.72	0.0001	1.09	0.0001	92.23	0.0001	8.72	0.0001
Temp*Rep(Iron)	40	0.08	1.0000	0.19	0.1453	1.15	0.7838	0.81	0.6325
Error	100	1.08		0.36		3.58		2.23	
Corrected Total	199								
C.V.		3.74		4.02		3.96		7.90	

<sup>a</sup>MSI = model system I; MSII = model system II.

<sup>b</sup>df = degrees of freedom.

<sup>c</sup>SS = sums of squares for type III estimable function.

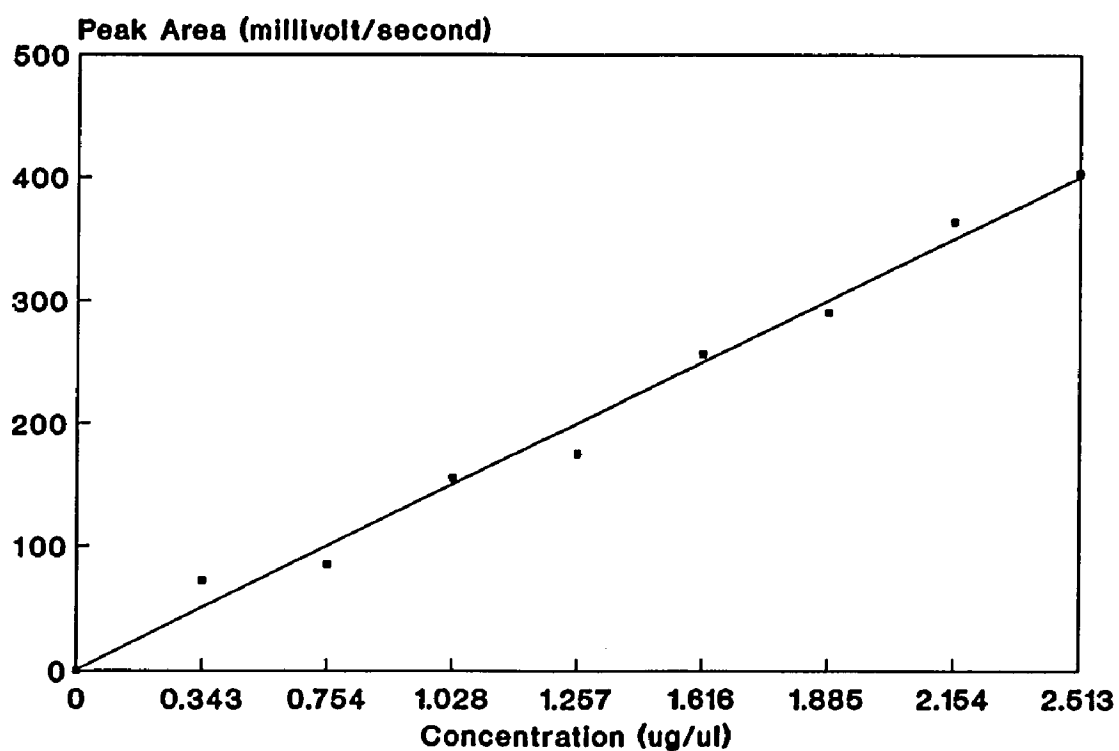
Appendix Table 6

Analysis of variance for PV in beef and chicken model systems I and II<sup>a</sup>.

Source	df <sup>b</sup>	Beef				Chicken			
		MSI <sup>a</sup>		MSII <sup>a</sup>		MSI <sup>a</sup>		MSII <sup>a</sup>	
		SS <sup>c</sup>	P>F	SS <sup>c</sup>	P>F	SS <sup>c</sup>	P>F	SS <sup>c</sup>	P>F
Iron	9	1775.5	0.0001	460.90	0.0001	2453.55	0.0001	1873.9	0.0001
Rep(Iron)	10	11.64	0.9534	20.58	0.9482	73.25	0.4270	48.35	0.5065
Temp	4	3452.7	0.0001	1683.4	0.0001	3808.34	0.0001	1595.5	0.0001
Temp*Iron	36	222.51	0.0035	20.32	1.0000	450.58	0.0154	129.25	0.8935
Temp*Rep(Iron)	40	110.13	0.6468	72.93	0.9999	238.30	0.7366	241.52	0.2674
Error	100	307.66		526.96		713.63		518.04	
Corrected Total	199								
C.V.		13.01		25.72		14.95		19.03	

<sup>a</sup>MSI = model system I; MSII = model system II.<sup>b</sup>df = degrees of freedom.<sup>c</sup>SS = sums of squares for type III estimable function.

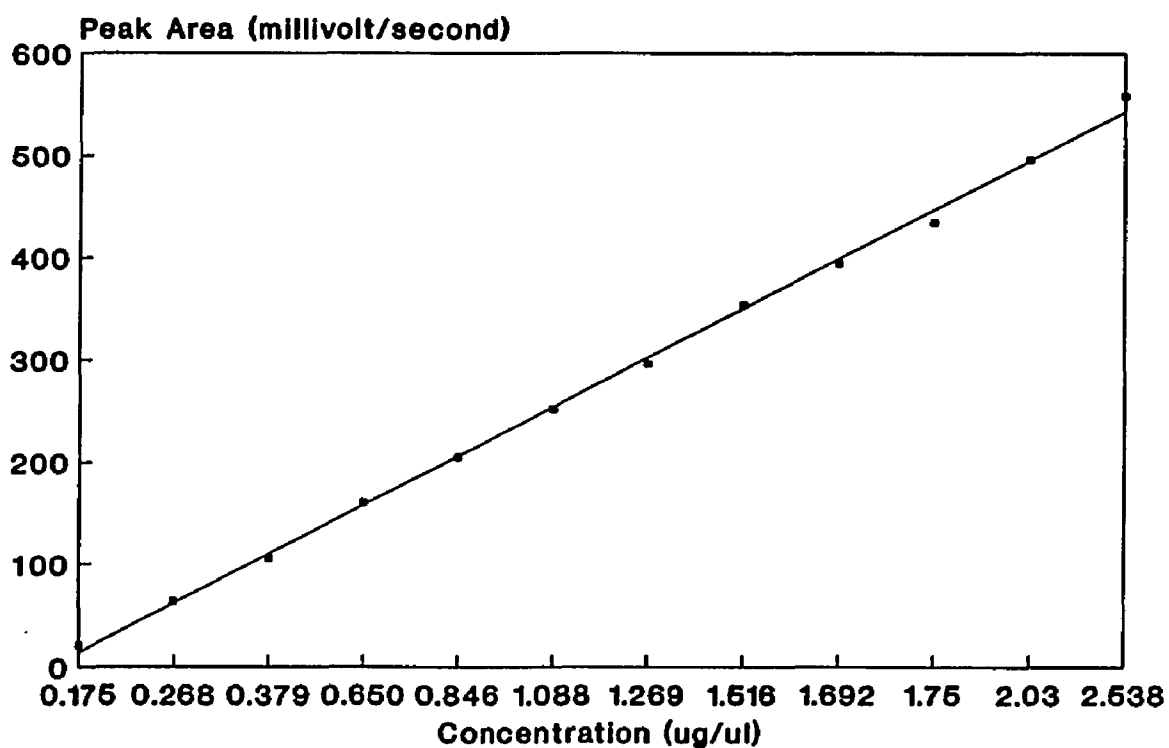
**Appendix Figure 1: Standard curve of myoglobin from size exclusion column used to calculate concentration of total pigment in samples.**



$$\text{Concentration} = (2.4538 \times 10^{-3}) * \text{Peak Area} + 2.192 \times 10^1$$

$$R^2 = 0.9865$$

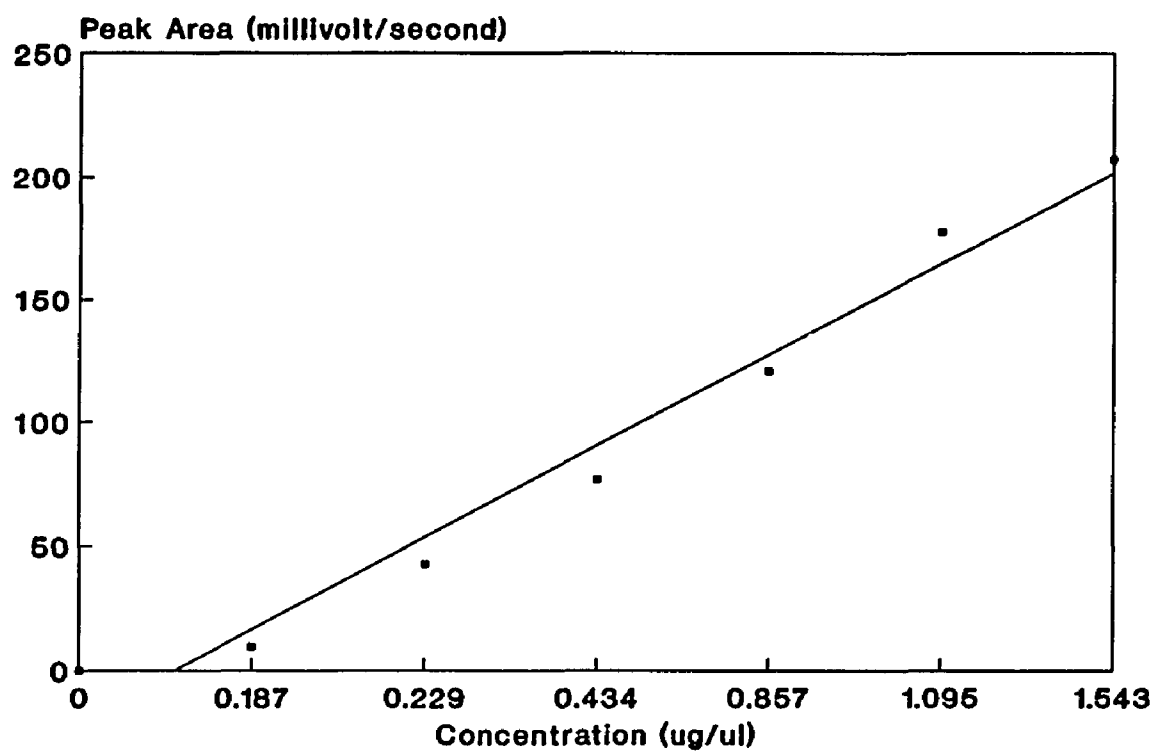
**Appendix Figure 2: Standard curve of myoglobin from hydrophobic interaction column used to calculate concentration of myoglobin in samples.**



$$\text{Concentration} = (1.7258 \times 10^{-3}) * \text{Peak Area} - 4.588$$

$$R^2 = 0.9947$$

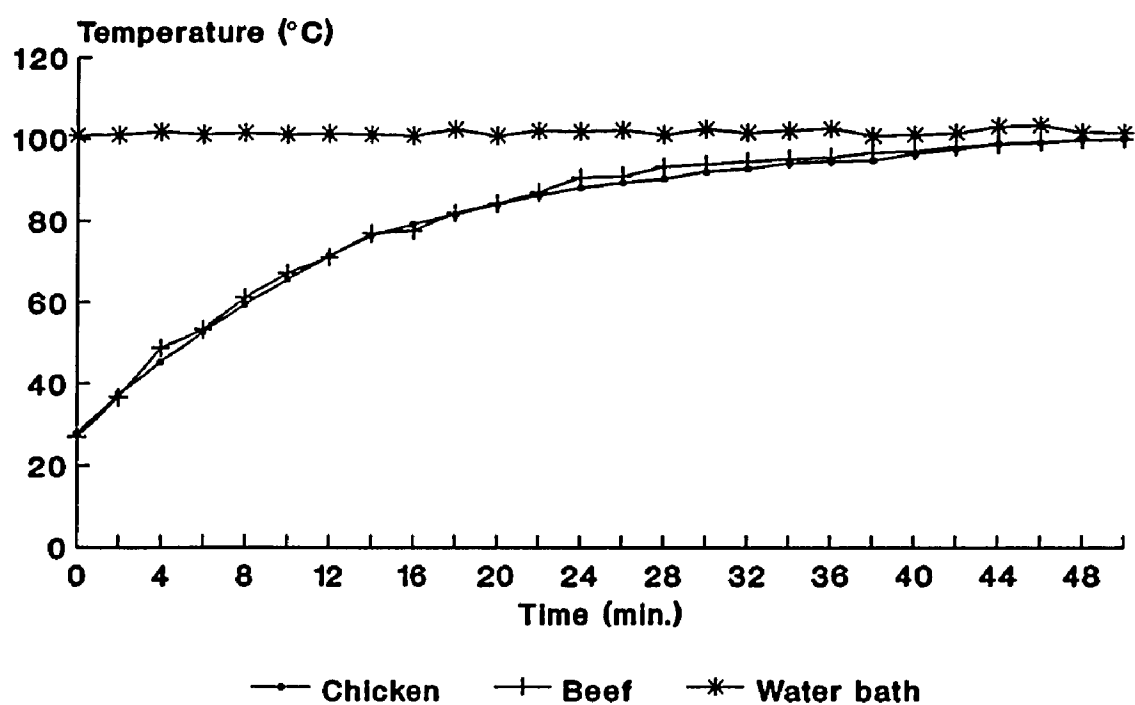
**Appendix Figure 3: Standard curve of hemoglobin  
from hydrophobic interaction column used to  
calculate concentration of hemoglobin in samples.**



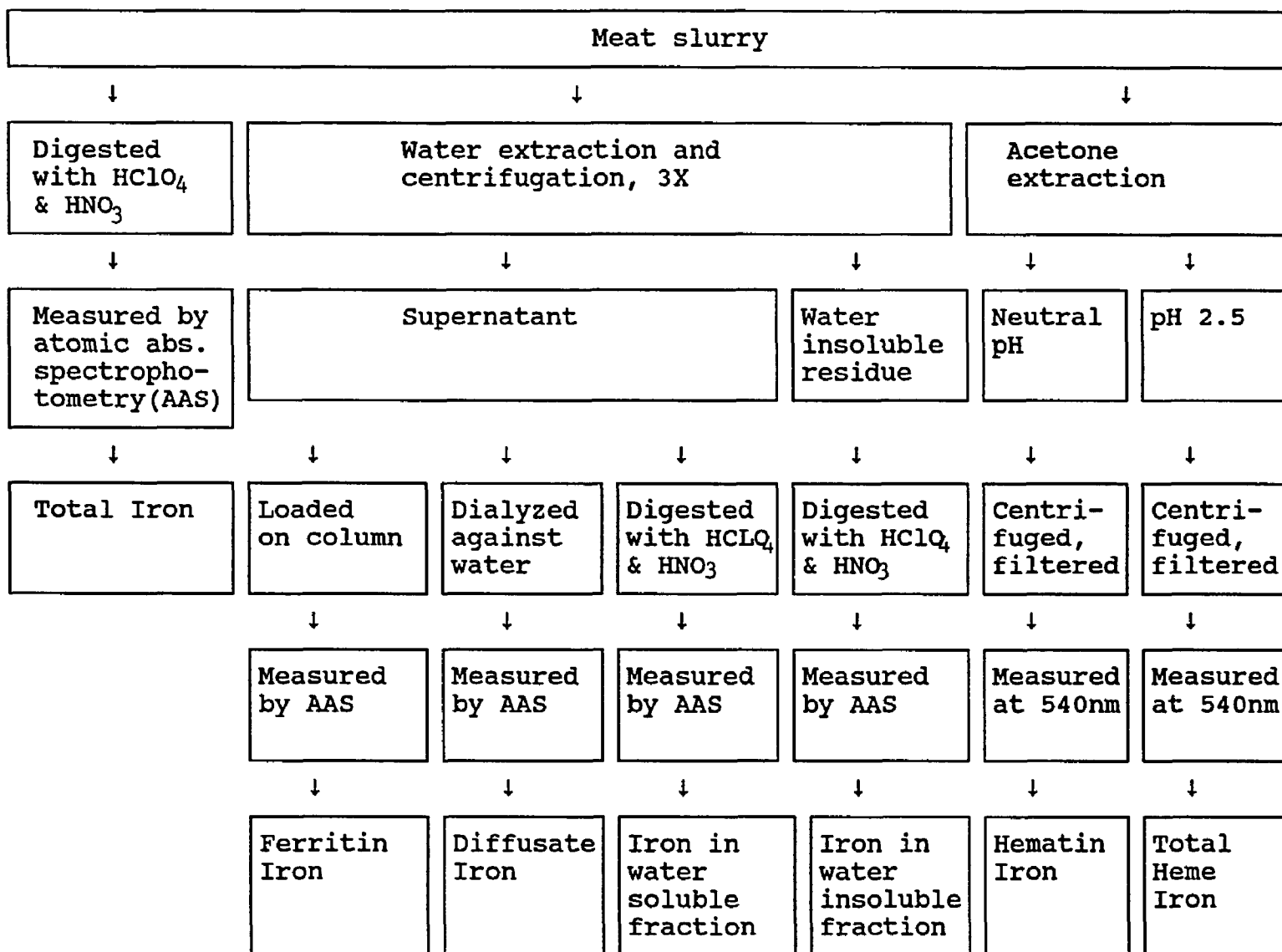
$$\text{Concentration} = (2.168 \times 10^{-3}) * \text{Peak Area} + 6.6094 \times 10^1$$

$$R^2 = 0.9811$$

**Appendix Figure 4: Heating rate of beef and chicken slurries in steam water bath.**

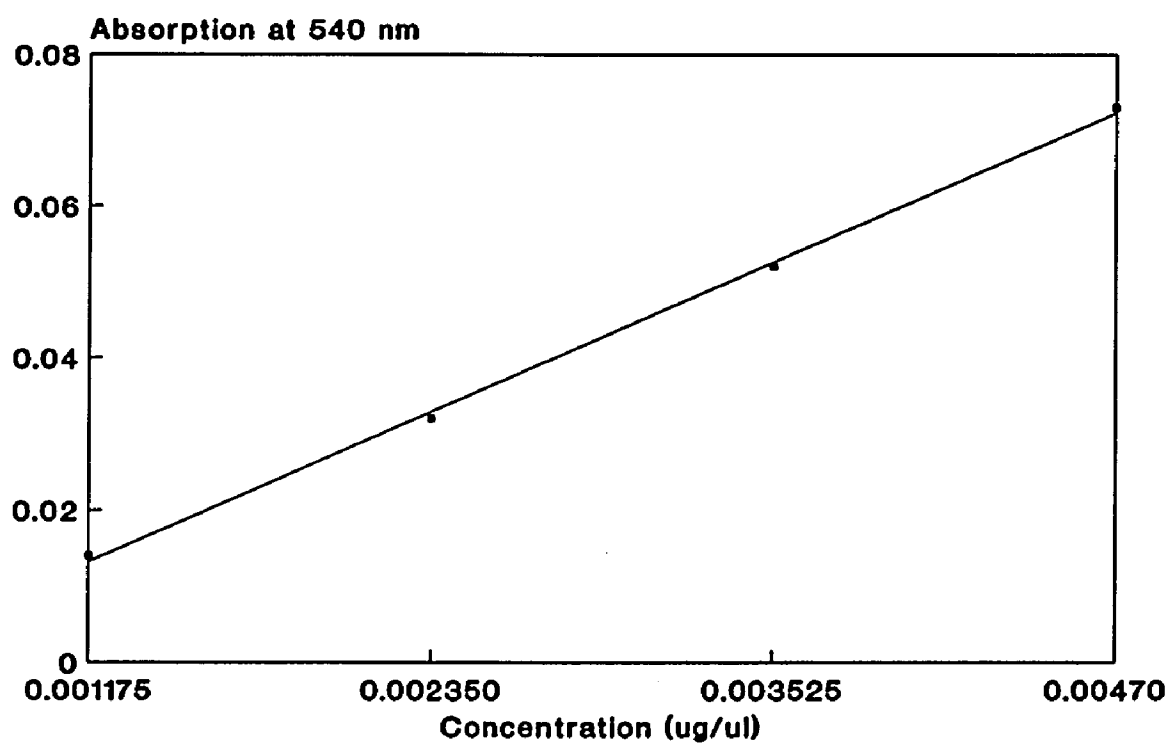


Appendix Figure 5: Determination of iron





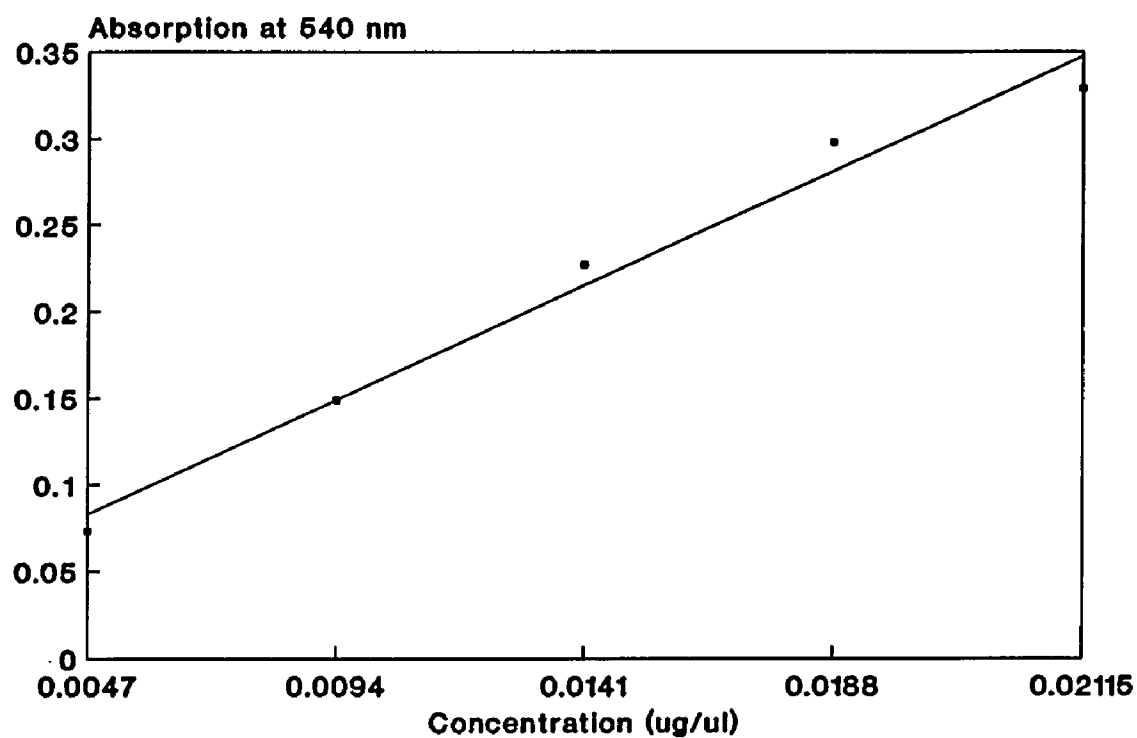
**Appendix Figure 6: Standard curve of hemin chloride used to calculate concentration of hematin in samples.**



$$\text{Concentration} = (5.964 \times 10^{-2}) * \text{Absorption} + 3.877 \times 10^{-4}$$

$$R^2 = 0.9988$$

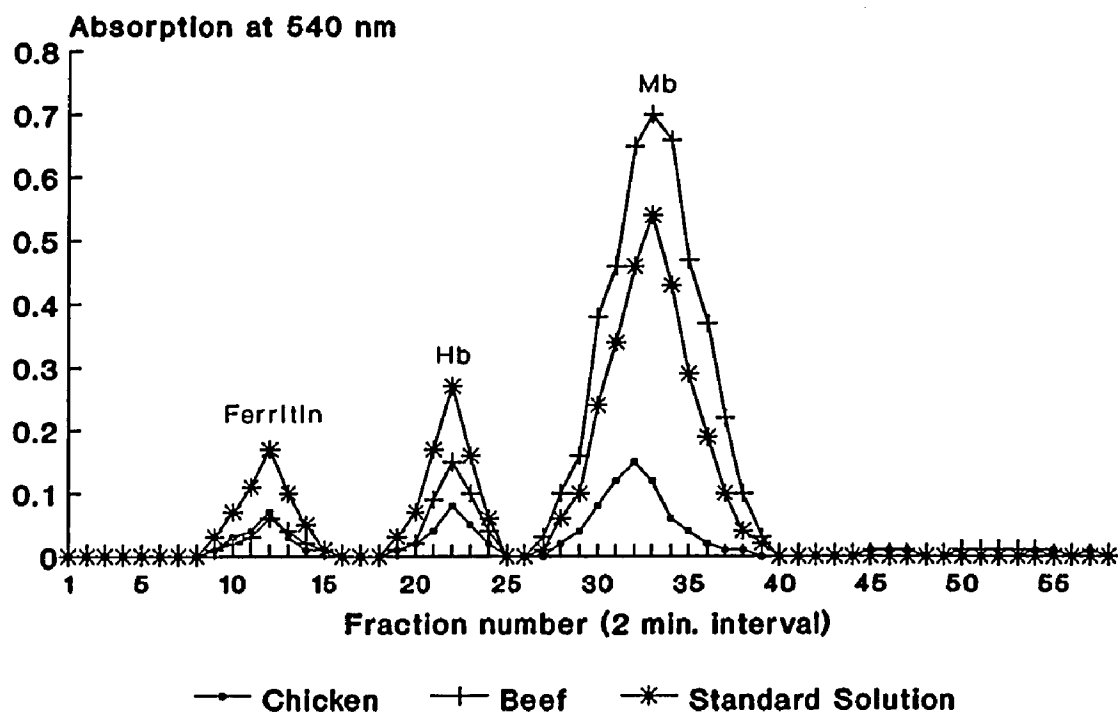
Appendix Figure 7: Standard curve of hemin chloride used to calculate concentration of total heme in samples.



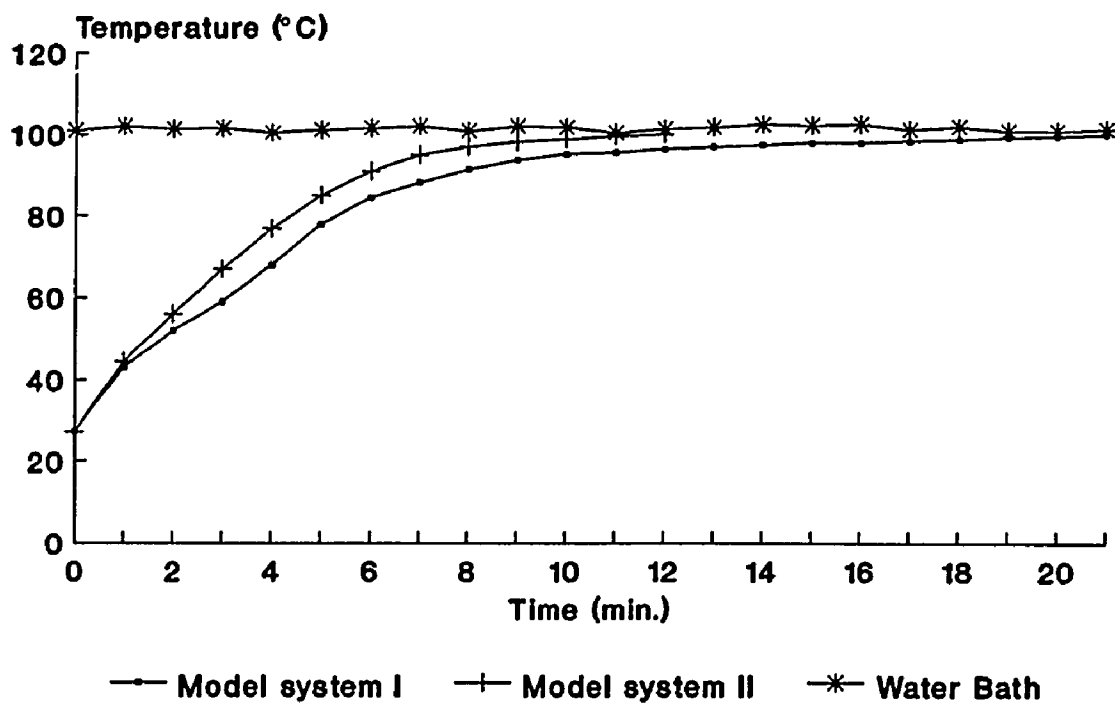
$$\text{Concentration} = (6.38 \times 10^{-2}) * \text{Absorption} - 9.92 \times 10^{-5}$$

$$R^2 = 0.999$$

**Appendix Figure 8: Chromatograms of the solution of ferritin, Hb, and Mb standards and aqueous extracts of beef and chicken muscles fractionated on Ultrogel AcA 34 gel filtration column.**



**Appendix Figure 9: Heating rate of meat residue (model system I) and fat emulsion (model system II) in steam water bath.**



## VITA

Dongmei Han is a native of Hangzhou, Zhejiang province, P.R. China. She is the daughter of Jingrong Han and Mingyu Yuan. She received her Bachelor of Science in Chemistry from Hangzhou University, China in 1982 and Master of Science in Analytical Chemistry from Hangzhou University, China in 1985.

For four years she was employed as Research Assistant in the Department of Poultry Science, and later in the Department of Animal Science at Louisiana State University. She was previously employed as an Instructor of Chemistry in the Department of Basic Science at Zhejiang Agricultural University, China.

Dongmei lives in Baton Rouge, Louisiana with her husband, Chi Zhang.

# DOCTORAL EXAMINATION AND DISSERTATION REPORT

**Candidate:** Dongmei Han

**Major Field:** Animal Science

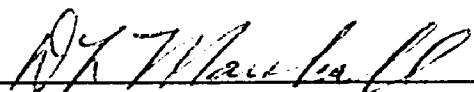
**Title of Dissertation:** Effects of Heat and Iron Fractions on Lipid Oxidation in Meat

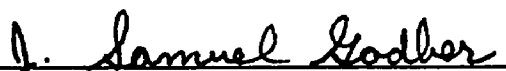
**Approved:**

  
Major Professor and Chairman

  
Dean of the Graduate School

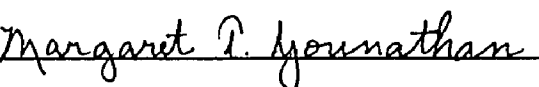
## EXAMINING COMMITTEE:











**Date of Examination:**

December 19, 1991